CRYPTOCOCCOSIS

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TORULOSIS OR EUROPEAN BLASTOMYCOSIS

by

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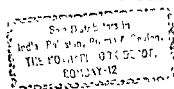
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Preface

With the ever-increasing importance of fungus diseases in the practice of clinical and laboratory medicine, a need has arisen for concise yet comprehensive and well-illustrated texts on the various mycoses In preparing this book on Cryptococcosis, our goal has been to produce such a volume in which the most useful features of a monograph and an atlas are combined. We have attempted to describe and illustrate fully the wide range of clinical and pathologic manifestations produced in man and animals by the fungus, *C neoformans*, and, at the same time, to provide historical, epidemiologic, and technical information for those interested primarily in these aspects of the subject. An appendix of the most im- [illegible]

conceived in 1948 after discussion with Brigadier General Raymond O. Dart, then Director of the Army Institute of Pathology. This plan received the [illegible]

being made in the field of medical mycology, a comprehensive pictorial and textual treatment of all mycoses in a single volume would be outdated in part by the time it was published The plan therefore was changed, and it is now our intention to publish a series of monographs on individual systemic mycoses This is the first of the series

Throughout the planning and preparation of this book, we have had the constant, enthusiastic support of Brigadier General Elbert DeCoursey, Director of the Armed Forces Institute of Pathology and his successor, Captain William M Silliphant, U S N We are deeply grateful to them for making available to us the unparalleled collection of clinical data, pathologic material, photographs, and x-ray films on file at the Armed Forces Institute of Pathology This material includes over 100 cases of cryptococcosis contributed from all parts of the world We are also indebted to Directors Dart, DeCoursey and Silliphant for having made it possible for us to utilize the vast resources of the AFIP and the aid of its many talented employees

This book represents the combined efforts of a great many people—too many, unfortunately, to mention all by name. There are the many contributors who willingly shared their clinical and laboratory experiences by submitting their valuable case material to the AFIP We have made every effort to acknowledge them by name, especially in legends of the illustrations We have also drawn considerably upon the cumulative experiences of our colleagues, especially those pathologists on the Staff of the AFIP, many of whom have written on this same subject One whose help was deeply appreciated is the late

Miss Mary Francis Gridley. Her interest in methodology and particularly in the histochemistry of fungi, her eagerness to help, and her friendly attitude will long be remembered. Several of the methods given in the Appendix are taken from Miss Gridley's "Laboratory Manual of Special Staining Technics."

Our sincere appreciation goes to Mr. Julius Halsman and his corps of expert photographers who prepared most of the photographs, and to Mr. Herman Van Cott and Mr. Roy Reeves, who helped us evaluate the engravers' reproduction of these fine photographs. Without the help of the Medical Illustration Service of the AFIP, this work would not have been possible. We wish to acknowledge the assistance rendered by Mr. L. P. Ambrogi and his laboratory technicians, Mrs. Gwendolyn M. Evans and her clerical group, Mrs. Helen K. Steward and her editorial staff, and, of course, our own devoted secretaries and typists.

Finally, we wish to express our gratitude to our publishers, Grune & Stratton, Inc., and their editors, for their interest in our work and for the help and cooperation given us during the preparation of this book.

THE AUTHORS

Contents

PREFACE . . . v

I. INTRODUCTION . . . 1

II HISTORICAL ASPECTS . . . 1

III. INCIDENCE . . . 3

IV. SOURCE OF INFECTION AND PORTAL OF ENTRY . . . 6

V. HUMAN CRYPTOCOCCOSIS CLINICAL ASPECTS . . . 8
1. Pulmonary Cryptococcosis . . . 8
2 Cryptococcal Infection of the Central Nervous System . . . 17
3. Involvement of Skin, Mucous Membranes and Adjacent Structures . . . 24
4. Involvement of Bones and Joints . . . 30
5 Involvement of Other Organs . . . 34
6. Association with Lymphoma and Leukemia . . . 35
7 Relation to Sarcoidosis . . . 36

VI. CRYPTOCOCCOSIS IN ANIMALS . . . 38

VII IMMUNOLOGY . . . 47
1. Antigenic and Chemical Structure of the Cryptococcus . . . 47
2. Serology in Diagnosis . . . 48
3 Skin Hypersensitivity . . . 51

VIII. PATHOLOGY . . . 53
1 Pulmonary Cryptococcosis . . . 64
2 Cryptococcal Meningitis . . . 71
3 Cryptococcal Encephalitis . . . 74
4 Space-Occupying Granulomas of the Central Nervous System 82
5 Involvement of Skin and Mucous Membranes 82
6 Cryptococcal Involvement of Other Organs 83

IX LABORATORY STUDIES 91
1 Cytology of the Fungus . . . 93
2. Direct Examination of Tissue Fluids and Exudates 96
3 Culture Studies 99
Sabouraud dextrose agar 101
Littman oxgall agar . . . 102
Brain-heart infusion blood agar 102
Littman liver-spleen glucose blood agar . . . 103
Colony appearance of *C neoformans* var *innocuous* . . . 103
Ascospore production 103
Temperature tolerance and heat resistance 104
4 Biochemical and Physiologic Reactions 105
Carbon auxanographic agar method . . . 105

Carbon assimilation broth technic 106
Nitrogen assimilation 106
Nitrogen auxanographic agar method 106
Nitrogen assimilation broth technic 107
Streak plate method for carbon and nitrogen assimilation test 107
Production of starch-like compounds 107
Oxygen requirement, gelatin liquefaction, urease formation . 108
5 Botanical Classification of *C. neoformans* and Asporogenous Yeasts 108
Synonymy 110
6 Pathogenicity, Virulence for Animals . . . 111
White Swiss mice 112
Rat 113
Guinea pig 114
Rabbit 114
Chick embryo 114
7 Histopathologic Diagnosis 116
8 Criteria for Identification of an Organism as *C neoformans* . 120
Cellular morphology 120
Colony formation 120
Oxygen requirement 120
Tolerance to 37°C . . . 120
Biochemical . . 120
Serologic . . 120
Pathogenicity . . . 120
Histopathologic 120

X TREATMENT AND PROGNOSIS . 121
1 Surgical Treatment of Localized Lesions 121
Pulmonary 121
Extrapulmonary 124
2 Nonsurgical Therapy 125
Physical agents . 125
Hyperthermia, 125 X-irradiation, 128
Chemotherapy 128
Iodides, 128 Antimonials, 129 Sulfonamides, 129 Diamidines, 130 Ethyl vanillate, 133 Esters of parahydroxybenzoic acid, 133 Thiosemicarbazones, 133. Thiocarbamate derivatives, 134 Thiourea derivatives, 134 Compound P-594, 134 Fatty acid derivatives, 134 B-diethylaminoethyl fencholate, 134 Naphthoquinones and related compounds, 134 Quinacrine, 135 Antihistaminic compounds, 135 Isonicotinic acid hydrazide, 135 Miscellaneous compounds, 135 Daraprim, 135 Chlorquinaldol, 135

Antibiotic therapy 136

Antifungal substances derived from bacteria, 137. Antifungal substances derived from fungi, 137. Antifungal substances derived from actinomycetes, 138. Nystatin, 139. Candicidin, 139 Ascosin, 139 Actidione, 139 Fungichromin, 143. Mycoticin, 143. Antifungal substances derived from higher plants, 143.

Vaccine therapy 145
Enzymes 145
Effect of hormones 146
Symptomatic treatment 146
3. Summary of Treatment . . 146

ADDENDUM
Ascospore Production 147
Antifungal Substances Derived from Actinomycetes . . . 147
Fungichromin 147
Mycoticin . . 148
Nystatin 148
Miscellaneous Compounds 149
Chloroquinaldol . . 150
Effect of Hormones . . 150
Histopathologic Diagnosis . . . 150
Immunology 151
Involvement of other Organs . 151
Source of Infection and Portal of Entry 151

APPENDIX (CULTURE MEDIA AND SPECIAL STAINS)
1 Sabouraud Dextrose Agar . . 152
2 Mycological Agar . . 152
3 Mycophil Agar . 152
4 Littman Oxgall Agar 153
5 Littman Medium . 153
6 Brain-Heart Infusion Blood Agar 153
7 Littman Liver-Spleen Glucose Blood Agar . 153
8 Yeast Morphology Agar 154
9 Carbon Assimilation Agar (Auxanographic) for Yeasts 155
10 Carbon Assimilation Broth (Yeast Nitrogen Base) 155
11 Nitrogen Assimilation Agar (Auxanographic) for Yeasts 156
12 Nitrogen Assimilation Broth (Yeast Carbon Base) 156
13 Henrici's Vegetable Juice Sporulation Agar for Yeasts, Modified 157
14. Carrot Infusion Sporulation Agar for Yeasts 157
15 Synthetic Agar Medium for Detection of Extracellular Starch Production by Yeasts 157
16 Hotchkiss-McManus Stain, Periodic Acid-Schiff Technic 158
17 Gridley Fungus Stain . 159
18. Mayer's Mucicarmine Stain 160

19. Brown and Brenn Gram Stain 161
20. Gomori's Methenamine-Silver Nitrate Technic (Modified) . . 162

REFERENCES 163

INDEX 183

COLOR PLATES

Plate I. Macroscopic Lesions in Cryptococcosis. *Facing page 64.*

Plate II *C. neoformans* in Tissue Sections. *Facing page 90*

Plate III Colonial Characteristics of *C. neoformans* and *C. neoformans* var *innocuous* *Facing page 104.*

Plate IV. Encapsulated Cryptococci Demonstrated by Special Staining Procedures *Facing page 116.*

I. Introduction

Cryptococcosis, known also as torulosis and European blastomycosis, is a mycotic disease of man and animals caused by *Cryptococcus neoformans* (*Torula histolytica, Cryptococcus hominis*). This budding, yeastlike, nonmycelial organism has a characteristic feature that is unique among pathogenic fungi—the production of a mucinous capsule in tissue and in culture. The fungus is widespread in nature, and reports of the disease it produces have come from nearly all regions of the world

Cryptococcus infection, thought to be airborne from a reservoir in the soil, may be disseminated hematogenously from the lungs to any part of the body. Involvement of the brain and meninges, which are particularly susceptible, is the usual cause of death. The tissue response to the organism varies from a sparse production of macrophages to formation of tuberculoid granulomas, while suppuration and caseation necrosis are distinctly unusual.

Prognosis is generally good unless the nervous system becomes involved, under which circumstance a fatal outcome is almost certain. This fact, together with the knowledge that there is presently no effective medical therapy, pointedly indicates the importance of early diagnosis and surgical excision of localized lesions

II. Historical Aspects

Busse[50, 52] and Buschke[49] (1894–1896) independently reported the recovery of a yeastlike organism from a 31 year old woman with a "gumma-like" or "sarcoma-like" lesion of the tibia, who also had lymphadenopathy and secondary skin lesions The knee joint subsequently became involved and the patient died with multiple lesions of the lungs, spleen, kidney, bones and skin Busse[51, 52] referred to the organisms as "Hefe" (Saccharomyces) and to the disease as saccharomycosis hominis. About this time (1894), Sanfelice[158-160] isolated from peaches a yeast which he named *Saccharomyces neoformans*, thus giving priority to the species term, *neoformans*

The following year, Curtis[86] recovered a pathogenic yeast from a myxomatous tumor in the hip of a patient and called the organism *Saccharomyces subcutaneus tumefaciens* Vuillemin,[440] recognizing that the lack of ascospore formation differentiated this organism from the true yeasts, adopted the name *Cryptococcus hominis* The generic term *Cryptococcus* had been used originally by Kutzing[227] to designate organisms thought to be algae In 1902, Frothingham[159] recovered the Busse-Buschke organism from a myxomatous pulmonary

lesion of a horse. In the same year, Weis[458] reported that two strains isolated by Sanfelice and Klein from, respectively, peaches and milk were microscopically and culturally identical with two recovered by Sanfelice and Plimmer from human "cancers." Later, von Hansemann[166] in Berlin reported a case of "tuberculous meningitis" in which small gelatinous cysts containing yeast cells were found in the meninges.

The first human case of nervous system cryptococcosis (European blastomycosis) said to have been correctly diagnosed ante mortem was reported by Versé,[437] who observed widespread leptomeningitis in a 29 year old woman. Shortly afterward, Stoddard and Cutler[407] reported two cases of their own, named the causative organism *Torula histolytica,* and described the clinical and pathologic differences between cryptococcosis, North American blastomycosis, and other fungus infections. The term, which still is used widely, was chosen because of an assumed "histolytic" action of the organism in forming cysts in tissue.

In 1935, Benham[23] made a systematic study of 22 strains of pathogenic cryptococci, including transplants of the original cultures of Busse, Buschke and Curtis, which fortunately were among those to be found in the American Type Culture Collection All strains proved to be biologically similar. Benham suggested retaining the name *Cryptococcus hominis* for the species

Throughout the years, classification of the organism has been controversial. Since *Cryptococcus neoformans* has consistently failed to form true mycelium or to produce ascospores, it has been grouped with anascosporogenous yeasts Todd and Hermann,[425] however, reported that the organism formed a structure which they interpreted as an ascus containing one ascospore, although they did not employ spore stains On this basis they recommended reclassification of the organism as *Debaryomyces hominis* in the ascosporogenous genus *Debaryomyces* Although a number of investigators confirmed this observation, others were of the opinion that the structure represented a modified capsule rather than a true ascus The consensus among medical mycologists at present is that the most valid name, on the basis of usage and priority, is *Cryptococcus neoformans,* with *Cryptococcus hominis* and *Torula histolytica* falling into synonymy. In the 1952 taxonomic study of the yeasts by Lodder and Kreger-Van Rij,[239] the organism was classified as *Cryptococcus neoformans* (See Botanical classification of *C neoformans* and asporogenous yeasts, page 108)

III. Incidence

ONCE CONSIDERED a rarity, cryptococcosis is now being recognized and reported with greater frequency. This is demonstrated graphically by the curve in Figure 1, which indicates the remarkable upsurge of interest in this disease subsequent to the papers of Levin[237] in 1937 and Binford[14] in 1941. Now that over 300 cases are on record, papers which appear deal with larger series or with particular aspects of the disease in place of the sporadic case reports that were seen in the earlier literature.

C. neoformans is the most frequent cause of mycotic meningitis in man The 300 or so cases of cryptococcosis reported in world medical literature do not reflect the true incidence accurately, since, in the United States alone during

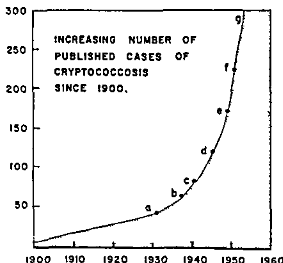


FIG 1—Number of reported cases of cryptococcosis has increased sharply during the last two decades, most likely due to an increasing interest rather than a greater incidence of the disease. *a*, Freeman[136] collected 43 cases in 1931, *b*, Levin[237] 60 cases in 1937, *c*, Binford[14] 70 cases in 1941, *d*, Cox and Tolhurst[49] 120 cases in 1946, *e*, Mosberg and Arnold[291] 172 cases in 1950, and *f*, Evans and Harrell[119] 221 cases in 1952. *g*, Over 300 cases were on record by 1955

a 4 year period after 1949, 151 fatal cases were recorded by the National Office of Vital Statistics.[435] Approximately 10 per cent of the 300 to 400 fatal mycoses reported annually in the United States are caused by *C neoformans* The actual world incidence of all mycoses cannot be estimated accurately by the number of published case reports, and must be considerably greater The increasing importance of mycotic disease in the United States is illustrated by the fact that the total deaths attributed to this group was greater in each year from 1949 through 1952 than the combined fatalities caused by rickettsial, protozoal, and helminthic diseases

Experience with cryptococcosis in a number of different hospitals provides some information as to the frequency with which the infection is recognized, but again these figures probably do not represent even a close approximation of the actual incidence There were four cases in 537,135 admissions to the Charity Hospital in New Orleans over a 10-year period[155] At the Johns Hopkins

Hospital in Baltimore, four cases were recognized over a period of 20 years,[121] and precisely the same incidence was reported at the Mount Sinai Hospital in New York by Globus and co-workers[150] There is good reason to believe that other cases remained unrecognized at these and similar institutions. For example, at the Mount Sinai Hospital, we have personal knowledge of four proved cases in a period of only 12 months during 1954 and 1955. It is also interesting to note that at the Duke University Hospital, where interest in medical mycology has been exceedingly high, 33 cases of cryptococcosis have been diagnosed clinically over a period of twenty-three and a half years, and pathologic diagnoses have been made in 12 autopsied cases[14]

Another reason for believing that many cases go unrecognized and unreported is that there is a considerable discrepancy between the clinical and pathologic diagnosis. The incorrect clinical impressions may be tuberculous or syphilitic meningitides, brain tumor, or metastatic carcinoma. Incorrect pathologic diagnoses of fatal cryptococcal meningoencephalitis are considerably less frequent *Additional fatal cases unrecognized as cryptococcosis are to be found* among patients with malignant lymphoma and leukemia. The fungus infection in such patients is almost invariably an overwhelming, widely disseminated process which, prior to autopsy, is completely overlooked, and the patient's death usually is attributed to his malignant disease At the Armed Forces Institute of Pathology, where over 60 fatal and 20 localized cryptococcal infections have been *filed, approximately one-third occurred in patients with a pre-existing* lymphoma or leukemia[475,476] In most of these cases cryptococcosis was not suspected before autopsy

In view of the fact that many cryptococcal infections are diagnosed only after autopsy and because only a small percentage of patients who die are autopsied, the actual incidence of cryptococcosis is undoubtedly much higher than that found in medical literature or in reviews of vital statistics

It is even more difficult to estimate the actual incidence of pulmonary cryptococcosis, particularly in the absence of dissemination The 20 or so cases of localized pulmonary lesions on file at the Armed Forces Institute of Pathology have all been contributed since 1950 Experience based on these cases indicates that pathologists have greater difficulty in arriving at the etiologic diagnosis of localized infections than in autopsied cases of cryptococcal meningoencephalitis. At times the organisms are not numerous and may be easily overlooked, with the result that the case may be diagnosed erroneously as "Boeck's sarcoid" or "granuloma of lung, etiology undetermined" Even though fungus cells may be seen, species identification based on histopathologic studies may prove inaccurate. In these cases, the fungus must be differentiated from *Blastomyces dermatitidis* and *Histoplasma capsulatum*

We know of no reliable data as to the frequency with which this fungus is responsible for minor, inapparent infections Skin testing programs that have demonstrated widespread contact with other micro-organisms—notably *Mycobacterium tuberculosis, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis and Toxoplasma gondii*—have not been feasible in this disease since a hypersensitivity state is thought not to develop Recent knowledge that thinly encapsulated cryptococci are antigenic (see Immunology, page 47) may

radically change this belief. Relatively asymptomatic pulmonary disease does occur as evidenced by the clinical histories and radiologic changes in patients in whom the organisms metastasize to the brain and meninges. Patients have been found to have pulmonary cryptococcosis while hospitalized for unrelated conditions (e g, pemphigus vulgaris in the case reported by Linden and Steffen[246]) and as a result they have been observed throughout the course of the pulmonary disease. Since some patients had minimal pulmonary symptoms, their infections would have been overlooked had they not been under close medical observation.

Of even greater interest are asymptomatic infections such as Weidman himself experienced. While at work in the laboratory in 1935 he coughed up sputum which on culture yielded an organism identified as *Torula histolytica* (*Cryptococcus neoformans*) He remained well and reported this experience 15 years later[433] Since pathogenicity studies and tolerance of incubation at 37°C apparently were not carried out, it is possible that the organism may have been the nonpathogen, *C neoformans* var. *innocuous*. The fact that patients harboring even sizeable pulmonary granulomas may be asymptomatic suggests that minor inapparent infections are much more common than we realize

IV. *Source of Infection and Portal of Entry*

CRYPTOCOCCOSIS has been reported from central Europe, Great Britain, Scandinavia, the Middle East, the Belgian Congo, South Africa, India, the Philippines, Indonesia, China, Japan, Hawaii, Central America, South America, Canada, the United States and Australia (the greatest number of cases from the last two countries). It is apparent that the disease is world-wide in distribution. Although the largest number of cases have been reported from the United States, highly endemic areas of this disease, comparable to those of coccidioidomycosis, histoplasmosis and North American blastomycosis, have not been found in this country.

According to the classification adopted by Lodder[257] in 1934, there were 25 species of cryptococci, of which all but *C. neoformans* were saprophytic and widespread in nature, being found on animal and vegetable material and foodstuffs. Emmons[105] found relatively avirulent species of cryptococci to be prevalent in soil and in a variety of rodents. The species in the genus *Cryptococcus* now have been narrowed down to seven (see page 110). Saprophytic varieties of cryptococci occur in great number on the skin and in the intestinal tract.[27,122] Studies by Ravits[331] revealed the presence of cryptococci on the skin of over half the normal subjects studied. We have isolated from the air and from sputum encapsulated saprophytic cryptococci which are morphologically indistinguishable from pathogenic strains, but they fail to grow at 37°C and are avirulent. These strains are now referred to as *C. neoformans* var. *innocuous*.*

Pathogenic strains of *C. neoformans* have been recovered from the oropharynx, normal skin, vagina, and gastrointestinal tract of man. Strains found on normal skin may be morphologically and culturally indistinguishable from *C. neoformans* except for their limited pathogenicity for mice and rats.[23] However, pathogenicity is relative, since cryptococci may gain virulence on serial passage through young rats. Benham studied some strains of cryptococci isolated from normal skin and found that they would produce splenic lesions only if inoculated into vitamin-deficient animals. This suggests that some human infections may be endogenous, resulting either from enhanced virulence of the organism or lowered host resistance. Such a theory seems particularly attractive to account for those infections developing during the course of other diseases, especially Hodgkin's disease and leukemia.[476]

Pathogenic *C. neoformans* was recovered from peaches by Sanfelice[335,336] in 1894. Although the fungus was also isolated from milk by Klein[211] in 1901 and by Carter and Young[56] in 1950, it was not again encountered as a saprophyte, unrelated to animal or human tissues, until 1951, when it was isolated from the

* Hereafter in the text, *C. neoformans* will denote the pathogenic species.

soil by Emmons.[105,107] In a further report on this work, Emmons[109] pointed out that the soil samples bore a curious and possibly significant relationship to pigeons. Eight of eleven specimens of pigeon manure collected in Gaithersburg, Maryland, yielded the fungus In a much larger study conducted in Loudoun County, Virginia, pathogenic strains of *C. neoformans* were isolated from 63 (57 per cent) of 111 specimens of pigeon nests and pigeon droppings obtained from 19 different premises in the county.[110] These observations recalled to Emmons the epidemics of pneumonitis presumed to be histoplasmosis in which pigeon manure was a prominent factor in the environment of the patients involved. He suggested that in the future, cryptococcosis be considered in the differential diagnosis of similar epidemics

The respiratory tract is considered to be the usual portal of entry of the organism, since many patients with cryptococcal meningitis give a history of recent respiratory infection. The observations by Terplan[422] and others[131,172] of focal cryptococcal lesions in the lungs and pleura of patients with cryptococcal meningitis lend further support to belief in the exogenous origin of the disease. Additional evidence is the discovery of small, discrete, subpleural nodules as incidental lesions at autopsies of patients who died of unrelated diseases

Some evidence does exist that other tissues, particularly skin and mucous membranes, may be sites of primary inoculation in man as well as in animals Freeman[135,136] pointed out that there is reason for believing the pharyngeal structures are the portal of entry in some cases, and stated that cryptococci have been recovered in pure culture from the tonsils In Semerak's two cases the oldest lesions were in the vicinity of the gasserian ganglion Together with Weidman, Freeman[137] demonstrated organisms in the meninges of guinea pigs within three weeks after intranasal inoculation Others, however, have experienced difficulty in infecting mice by this route[13,441] (see Pathogenicity, Virulence for Animals, page 111)

There are no reported instances of transmission of the disease to man from man or animal, although naturally occurring infections have been noted in many mammalia

Recently there have been extensive outbreaks of cryptococcal mastitis in herds of dairy cattle, but no human cases were reported among the personnel caring for the herds[103,199,324,386] Because of the heat-sensitivity of the organisms they would not survive either the holding or flash process of pasteurization (see Table I, page 46)

Cryptococcosis has been described in patients of all ages Two-thirds are between the ages of 30 and 60 years Males are somewhat more frequently affected than females Most of the cases have occurred in white individuals The greater susceptibility of dark-skinned races observed in coccidioidomycosis and North American blastomycosis is not apparent in cryptococcosis. Geographic location and occupation seem to play little part in the incidence of the disease

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FIG 2 —Localized subpleural granulomas interpreted as primary sites of cryptococcal infection *A* This nodule from the lower lobe of the right lung was an incidental autopsy finding in a 78 year old woman who died of calcific aortic stenosis Large numbers of cryptococci were identified in the central portions of the granuloma × 8 Case of Haugen and Baker [172] Courtesy of Dr R D. Baker, Professor of Pathology, Duke University, School of Medicine, Durham, N C *B* A healing cryptococcal granuloma obtained by biopsy at exploratory thoracotomy, proved to contain viable *C neoformans* by culture and microscopic examination The patient died of disseminated tuberculosis 7 months later Autopsy revealed no evidence of active cryptococcosis. × 24 (AFIP Accession No 510705, contributed by Veterans Administration Hospital, Memphis, Tennessee)

than 1.5 cm in the greatest diameter may be found at autopsy of patients who have died of unrelated diseases [172]

Large pulmonary lesions associated with nervous system involvement are readily detected by x-ray studies (Fig 3) and a specific diagnosis may be established by culture of sputum or bronchoscopic aspirate[167,426] or by bronchoscopic biopsy [317] Aspiration biopsy of the lung may establish the correct diagnosis,[94] but this seems to be an unnecessary and dangerous procedure In some cases rather large pulmonary lesions have been shown to regress or disappear completely before the onset of symptoms of cerebral metastasis [164]

Pulmonary cryptococcosis has also been discovered in the absence of nervous system involvement, and in rare instances has been responsible for the patient's death [289,381,423] A few patients with pulmonary cryptococcosis who were treated medically were said to be cured or at least were asymptomatic at the time of reporting.[66,155,167,240,304] Surgical extirpation suggested by Taber[416]

V. Human Cryptococcosis
Clinical Aspects

CRYPTOCOCCUS INFECTION has become almost synonymous with meningoencephalitis for two important reasons First, there seems to be a remarkable tissue susceptibility of the central nervous system and its meninges Second, involvement of the lungs and other tissues may not be productive of important symptoms or may be overlooked in the face of obvious meningoencephalitis Fatalities are almost invariably the result of spread to the brain and meninges Hence, the most important reason for early recognition and treatment of localized lesions is the prevention of metastatic involvement of the nervous system.

Occasionally the disease may be limited to a relatively localized area in the lungs, lymph nodes, skin, bone, eye, brain, or spinal cord. Such cases, some of which are amenable to excisional therapy, apparently are being encountered much more frequently today than in former years. For example, at the Armed Forces Institute of Pathology, more than one fourth of the 80 cases of human cryptococcosis on file are localized infections, and all but one of these have been received during the last five years

In the past it has seemed that widely disseminated infections were much less frequently encountered in cryptococcosis than in coccidioidomycosis and North American blastomycosis However, with thorough study, evidence of dissemination can be demonstrated histologically in at least half the autopsied cases, and in those infections complicating a pre-existent malignant disease of the reticuloendothelial system, dissemination is present in almost every case

Recently it has been reported that a disease in the newborn with striking similarity to toxoplasmosis may be caused by *Cryptococcus neoformans*[174,294,299] We, ourselves, have not observed the characteristic triad of chorioretinitis, intracranial calcification and meningoencephalitis as a result of proved congenital or neonatal cryptococcosis More convincing data are required before these reports can be accepted

1 PULMONARY CRYPTOCOCCOSIS

Although it is generally thought that the lungs serve as the portal of entry, some have objected to this belief on the grounds that many published autopsy reports of cryptococcal meningoencephalitis have not included description of pulmonary lesions Others, however, have noted that the primary focus in the lung may be small and readily overlooked[131 172 422] (Fig 2) These small lesions do not produce significant symptoms nor do they produce radiologic shadows, hence they are missed by clinician, radiologist and pathologist alike, unless specifically sought Isolated, discrete, subpleural granulomas measuring less

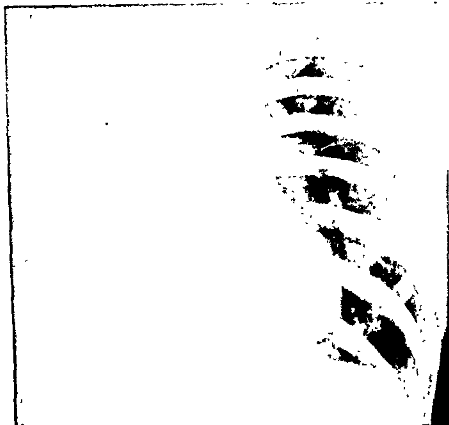


FIG. 4 —Extensive pleural effusion of six months' duration in a 34-year-old male Japanese-American veteran whose acute illness began with chills, fever, and chest pain Repeated attempts to demonstrate tubercle bacilli in sputum, pleural fluid, gastric washings, and urine were unsuccessful. One year after onset, the patient died of cardiac arrest (pontocaine sensitivity) during preparation for bronchoscopy Autopsy revealed the right lung to be firmly adherent to the parietal pleura and diaphragm Multiple plaque-like nodular granulomas measuring from 0 1 to 2 0 cm in diameter were present in the adherent pleural layers and in the subpleural parenchyma On microscopic examination, lesions containing numerous typical mucicarmine-positive cryptococci were seen There was no evidence of dissemination (AFIP Accession No 480669)

in 1937 has been reported by others,[30,93,94,139,268,314,320,363,411] and in the case originally reported by Froio and Bailey, an 8-year cure was obtained.[172] In one case on file at the Armed Forces Institute of Pathology, the patient was well 4 years after endoscopic resection of a bronchial granuloma. Prolonged follow-up is essential if claims of cures are to be accepted, as illustrated by Palmrose and Losli's[314] first case in which death occurred 3 years after the pulmonary lesion was resected

There is little that is characteristic about the signs or symptoms of pulmonary cryptococcosis, and, in fact, many cases are asymptomatic. Symptoms of pulmonary cryptococcosis when present are cough, scanty mucoid sputum, and in-



C

FIG 3.—Discrete pulmonary granuloma due to *C neoformans* first detected during a routine physical examination when patient was asymptomatic The lesion was not treated surgically About two and a half months after admission the patient first noted headache, and within a month died of meningoencephalitis There was insignificant change in the chest x-ray films during this three and a half month period *A* Discrete but not sharply circumscribed lesion in the right lower lung field *B* Same lesion demonstrated by laminograph *C* Lungs obtained at autopsy, sectioned longitudinally and the posterior halves removed The pulmonary granuloma (arrows) lies beneath the pleura but occupies much of the middle lobe of the right lung Pleural reaction is minimal (See also Fig 50*A*) Case reported by Ratcliffe and Cook[29] (AFIP Accession No 268527.)

Mediastinitis and direct extension to the neck or to the chest wall may occur,[304] but these are unusual complications. Some asymptomatic infections are detected only by x-ray examination.

Roentgenograms of pulmonary cryptococcosis show considerable variation. The most common lesion appears to be a solitary, moderately dense area of in-

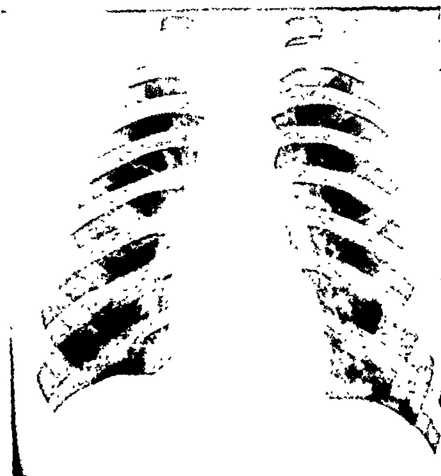


FIG. 6—Multiple discrete and confluent densities are present in the lower half of both lung fields. There is slightly increased prominence of the bronchovascular markings in the hilar areas (AFIP Negative No 218634–10 Courtesy of the Department of Radiology, The Charity Hospital of New Orleans)

filtration in the lower half of the lung fields, varying in size from 2 to 7 cm (Fig 3) In time, the infiltration slowly extends peripherally simulating neoplasm, lung abscess, or hydatid cyst However, there is little or no hilar enlargement. In some cases there is virtually no roentgenographic change (Fig. 5) or the lesion may regress and disappear [184 412] These solitary lesions are amenable to surgical extirpation (see Surgical Treatment of Localized Lesions page 121). Occasion-

frequent hemoptysis. Occasionally a cryptococcal focus may erode a bronchus and rupture into it to cause a heavy discharge of mucoid sputum containing large numbers of cryptococci. There may be, in addition, low grade fever, pleuritic pain, malaise or weight loss, but only rarely are these symptoms prominent. *In contradistinction to tuberculosis, night sweats seldom occur.* The course of pulmonary cryptococcosis is usually subacute or chronic and is



Fig 5—A small area of infiltration in the right upper lung field was observed over a 16 month period with essentially no change in appearance When the patient developed hemoptysis, the lesion was resected *A* X-ray, November 1952, *B* x-ray, April 1954, prior to surgical resection of involved lung See Fig 47 for unusual histopathologic features of this case (AFIP Accession No 654575, contributed by Valley Forge Army Hospital)

accompanied by few local or constitutional signs and symptoms. Occasionally the pulmonary manifestations may be severe, such as in the fulminant case of Sheppe's[381] which was accompanied by fever and signs of pulmonary consolidation, and which proved to be fatal 2 months after onset

Physical examination reveals signs of bronchitis or pulmonary consolidation Dullness to percussion and diminished breath sounds may be found. When miliary lesions are present, moist râles may be heard over the lung apex or at the base A pleural friction rub may be detected Pleural effusion is infrequent, but when it is present (Fig 4) a specific diagnosis may be established by thoracentesis and direct examination or culture of the withdrawn fluid[155,322]

tends even to the gross appearance of the pulmonary lesions. On histological examination, however, they appear early as masses of cryptococci with little inflammatory reaction or later as granulomas, and therefore have been called gelatinous or granulomatous miliary tubercles by Baker and Haugen.[15]

Coin lesions of the type characteristic of coccidioidomycosis and histoplasmosis are rarely observed in cryptococcosis[473,474] Thin-walled cavities are not



FIG 8—Widespread bilateral peribronchial infiltration Case 1 reported by Collins[16] (AFIP Accession No 537918 Courtesy of the Department of Radiology, Presbyterian Hospital, New York City)

formed Rarely, small areas of radiolucency representing cavities may be seen within the dense pulmonary nodules (Fig 11) Shadows fanning out from the lung root and mimicking bronchogenic carcinoma are rarely encountered in this disease and are more characteristic of North American blastomycosis, while pleural reaction is more marked in actinomycosis

Judging from the marked roentgenologic variations in pulmonary cryptococcosis, it is necessary to include it in the differential diagnosis of pulmonary diseases of slow evolution such as primary and secondary carcinoma, sarcoidosis,

ally these relatively well defined nodules may be multiple, in which case they resemble metastatic neoplasms (Fig. 6).

A second type of pulmonary lesion is a broader, more diffuse pneumonic infiltration usually involving the lower half of the lung fields[184] (Fig. 7). Infiltrations increase in extent and when this occurs, bronchovascular markings and small nodular shadows are sometimes accentuated. Healing may result in slight residual fibrosis.[155] Although most cases show minimal pleural reaction, in



FIG 7—Pneumonic consolidation, pleural thickening, and atelectasis, left lower lung field, accentuated bronchovascular markings, right lower lung field (AFIP Negative No 218634–23 Courtesy of the Department of Radiology, The Charity Hospital of New Orleans)

others the parenchymal lesion may be obscured by pleural thickening and effusion (Fig 4) Organizing bronchopneumonia has also been described[381]

A third type represents a more extensive peribronchial infiltration which may be bilateral (Fig 8) These may progress to form woolly shadows,[83] and may become widespread and massive resembling those of advanced tuberculosis (Fig 9) Caseation necrosis or cavitation seldom occurs and fibrosis is minimal or absent Hilar lymphadenopathy is not conspicuous, and there are no areas of pulmonary collapse or calcification

When the cryptococcus disseminates, as is often the case in patients with pre-existing malignant lymphoma or leukemia, it is likely to produce a fourth radiologic type appearing as widespread minute lesions of the lungs, indistinguishable from miliary tuberculosis (Fig. 10). The striking similarity ex-

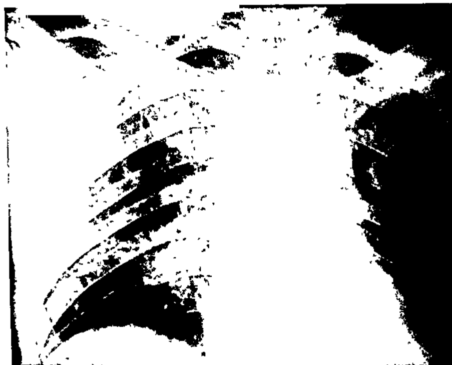


FIG 10—Miliary densities are present throughout both lung fields Patient died of cryptococcal meningoencephalitis and renal lesions containing cryptococci were found at autopsy *Microscopic examination of the lungs and mediastinal lymph nodes revealed widespread* involvement by non caseating epithelioid tubercles identical with those of sarcoidosis (Fig 44) Cryptococci were not demonstrable in the pulmonary lesions (AFIP Accession No 606695, contributed by Veterans Administration Hospital, Atlanta, Georgia)

2 CRYPTOCOCCAL INFECTION OF THE CENTRAL NERVOUS SYSTEM

Manifestations of nervous system involvement may begin insidiously without prodromal symptoms A history of a recent upper respiratory or pulmonary infection may be obtained Headache is often the first complaint and is intermittent at onset, later becoming continuous and progressively more severe The patient may be stricken suddenly with vertigo, faintness, vomiting and violent agonizing headache The pain appears most frequently in the frontal and temporal regions, over the sinuses or behind the eyes, and least frequently in the occipital area Patients often present themselves with tightly closed eyes and grimly drawn mouth, groaning involuntarily on movement This severe form of headache is usually associated with widespread cryptococcal invasion of cerebral tissues [135 136] The histories of most patients are dominated by headache

General physical and neurologic examination of the patient reveals a primary disturbance of the central nervous system and suggests meningitis or an expanding intracranial lesion If chronic respiratory infection or lymphadenopathy is also present, a tentative clinical diagnosis of tuberculous meningitis may be

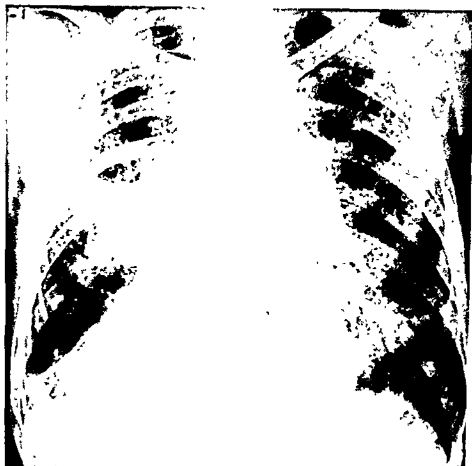


FIG 9—Extensive bilateral pulmonary cryptococcosis simulating far advanced tuberculosis (AFIP Negative No 218634-24 Courtesy of the Department of Radiology, The Charity Hospital of New Orleans)

hydatid cyst, chronic pulmonary tuberculosis, chronic lung abscess, bronchiectasis, pneumoconiosis and fungus infections other than cryptococcosis In differentiating pulmonary cryptococcosis from other diseases, 5 radiologic characteristics are important

(1) Predilection for the lower half of the lung fields.
(2) Rare cavitation.
(3) Minimal or absent fibrosis or calcification
(4) Inconspicuous hilar lymphadenopathy
(5) Infrequency of massive pulmonary collapse.

In the diagnosis of pulmonary cryptococcosis, recovery of the etiologic agent from the sputum is just as important as in pulmonary tuberculosis, i e , pathogenic *C neoformans*, like the acid-fast bacillus, is normally absent from the sputum, and when found there, permits a definitive diagnosis It is imperative, therefore, that any encapsulated yeast recovered from a source in the body other than the central nervous system be identified precisely, and that it fully satisfy the criteria for identification of *C neoformans* (see page 118).

weight and strength unless resort to forced feeding is made. The patient may have difficulty in swallowing and may be unable to take medication orally. Bowel and bladder disturbances are infrequent since cord lesions are rare Symptoms of acute infection and toxemia are usually absent.

The usual course of cryptococcal meningitis is one of progressive deterioration until death supervenes, but it is slower than that of tuberculous meningitis.

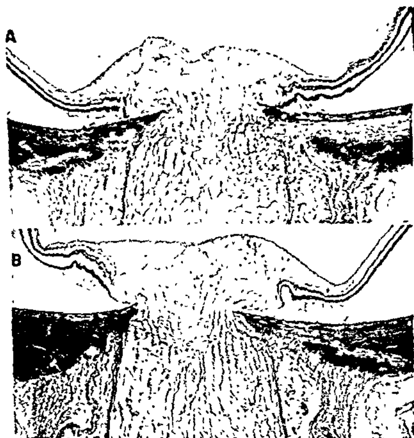


FIG 12 —Bilateral papilledema in a patient with cryptococcal meningoencephalitis A Right eye × 20 B Left eye × 20. (AFIP Accession No 176358, contributed by Station Hospital, Fort Bragg, N. C)

However, there may be a static period during which the infection seems to be arrested and the symptoms abate The periods of remission have led to erroneous conclusions regarding the beneficial effect of various therapeutic agents employed during the exacerbations Beeson[21] reported the case of a young woman who had repeated exacerbations and remissions of cryptococcal meningitis over a period of almost 16 years during which time she completed 2 successful pregnancies and had 2 abortions Her death was the result of marked meningeal thickening and hydrocephalus (Fig. 14). As a result of his experience, Beeson concluded that in view of the great chronicity and tendency to

offered. However, there is but little fever and the pulse and blood pressure are *often unaltered. Nuchal rigidity is a common sign and is occasionally associated* with tenderness of the neck, back and skull. Kernig's and Brudzinski's signs are frequently positive. Marked papilledema (Fig. 12) and retinal exudates are common, due to the greatly increased cerebrospinal fluid pressure. Ambylopia may be the result of this, or a lesion of the optic nerve[137] (Fig 13) Diplopia, strabismus, nystagmus, anisocoria, ptosis and loss of pupillary reactivity to light are frequently observed. Other ocular disorders recorded by



Fig 11 —A fairly well outlined, discrete area of consolidation is present in the left lower lung field In the center of the lesion is a loculated zone of diminished density interpreted *as early cavitation The patient died as a result of extensive cryptococcal meningoencephalitis (see Fig 55) At autopsy, a firm irregular granuloma* was found in the middle of the left lower lobe (see Fig 49) On macroscopic examination, small foci of necrosis with cavitation were seen (AFIP Accession No 270523, contributed by Veterans Administration Hospital, Alexandria, Louisiana)

Weiss, Perry and Shevky[437] were photophobia, neuroretinitis, retinal hemorrhages, primary optic nerve atrophy and ophthalmoplegia

In older patients mild changes in the sensorium may appear, such as irritability, restlessness, forgetfulness, apathy and confusion, but rarely disorientation Patients may be talkative or abnormally uncommunicative Disturbances of sleep, either somnolence or insomnia, are frequent Tinnitus is rare and deafness occurs only in the late stages Paresthesias are unusual and hemiparesis and hemiplegia are often later manifestations Tendon reflexes are variable and hyperreflexia is occasionally seen, but patellar and Achilles reflexes are often diminished or absent. *Anorexia is common* and results in pronounced loss of

hemorrhage, and uncinate hernia. Appropriate neurosurgical procedures may give symptomatic relief, and in rare instances of localized lesions amenable to complete extirpation, "cures" have been reported.[233, 241]

Roentgenographic findings in the skull may suggest an expanding intracranial lesion (Fig 15). Increased convolutional markings, suture separation and atrophy of the dorsum sellae and clinoids will suggest intracranial hypertension. The pineal gland and choroid plexus may be displaced. In a series of 31 cases of cerebral cryptococcosis, x-rays of the skull were abnormal in 13 (42 per cent)[60]

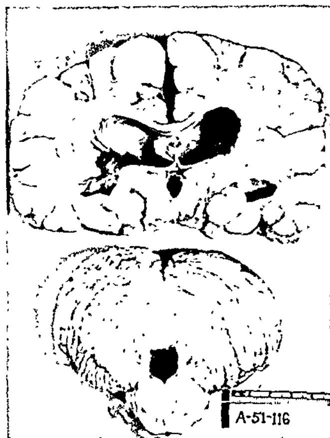


Fig 14—Extensive brain damage and internal hydrocephalus due to chronic cryptococcal infection, all four ventricles are widely dilated Case reported by Beeson.[22] (Photographs obtained through the courtesy of Dr W H Sheldon, Professor of Pathology, School of Medicine, Emory University)

Differential diagnosis in cases of nervous system cryptococcosis depends on whether involvement is diffuse or localized In the more frequent cases of generalized cryptococcal meningoencephalitis the clinical picture resembles tuberculous meningitis closely and the spinal fluid changes may also be indistinguishable except by cultural means When roentgenographic changes in the chest are suggestive of tuberculosis (Fig 9), the clinical diagnosis of tuberculous meningitis is given added support However, if the x-ray suggests bronchogenic or metastatic carcinoma (Figs 4, 6, 7 and 11), the neurologic manifestations may be interpreted to be due to metastatic tumor In such cases spinal fluid ple-

spontaneous remissions, caution should be exercised in accepting reports of "recovery" in patients with cryptococcic meningitis.

Eventually signs of increased cerebrospinal pressure appear, and bilateral papilledema or neuroretinitis becomes pronounced. Tremors and ataxia are uncommon and are late manifestations. In almost every instance cerebrospinal fluid pressure is increased, often to 700 mm. water. The patient becomes unresponsive and drifts off into sleep or semicoma from which he is aroused with difficulty. He seldom becomes violently excited and more often sinks into a low muttering delirium. When spinal or ventricular puncture is performed for

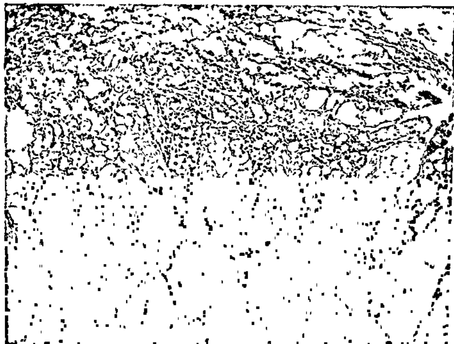


Fig ... from the brai... pial septae

removal of fluid, the reduction in intracranial pressure may restore the patient to consciousness and coherence, but he soon sinks back into deepening coma. He may have occasional attacks of Jacksonian epilepsy.

The moribund patient has an elevated temperature and pulse rate and usually exhibits flaccid paralysis of the extremities. Superficial and deep reflexes disappear. Eventually the respiratory center is affected and respiration ceases. Although there are fulminant cases in which the patient dies of meningoencephalitis in 2 weeks or less,[347] the usual duration is approximately 6 months. Chronic infections lasting a number of years are also on record.[21,337,439]

A study of 178 case reports of cryptococcal infection of the central nervous system by Carton and Mount[40] disclosed that surgical procedures had been carried out in almost one-fourth of the cases because of clinical indications of an expanding intracranial or intraspinal lesion. In such cases the preoperative diagnoses may include neoplasm, abscess, subdural hematoma, subarachnoid

The diagnosis of neurosyphilis may be suggested when by coincidence routine blood and spinal fluid serologic tests for syphilis are positive, and especially when such serologic evidence is coupled with a colloidal gold reaction of a strikingly "paretic" or "tabetic" type of spinal fluid In reviewing case histories of cryptococcal meningitis, one is struck with the frequent reference to benign lymphocytic choriomeningitis as the first clinical impression, however this virus disease is differentiated by its short and benign clinical course and by the absence of fungus cells in the spinal fluid. Rarely does cryptococcal meningitis pursue a fulminant course, but when it does, it may be mistaken for bacterial meningitis.



FIG 16—Hemorrhagic cryptococcal granuloma in posterior half of right cerebral hemisphere, meninges appeared normal. (Courtesy of Professor W St C. Symmers, Charing Cross Hospital Medical School, London, England, his Case 1, published in 1953 [41])

In older individuals with arteriosclerotic or hypertensive cardiovascular disease who have contracted cerebral cryptococcosis, the development of neuropsychiatric symptoms mistakenly may be attributed to multiple small cerebrovascular thromboses or hemorrhages.

When the cryptococcal granuloma is localized in the brain or spinal cord, the symptoms and neurologic signs produced are usually suggestive of tumor, abscess, subdural hematoma, or uncinate hernia (Fig 16) Diagnosis by spinal fluid examinations is less readily established than when the process is a *diffuse* meningoencephalitis In cases of this type frequently the correct diagnosis is made only after the neurosurgeon has resected or biopsied the lesion

Spinal fluid pleocytosis may or may not be present The meningitides of North American blastomycosis, coccidioidomycosis, actinomycosis, nocardiosis and moniliasis are differentiated reliably only by recovery of the causative organism from the spinal fluid or other tissues

Localized granulomas of the brain or spinal cord can occur with or without leptomeningitis On the other hand, the picture of an expanding intracranial

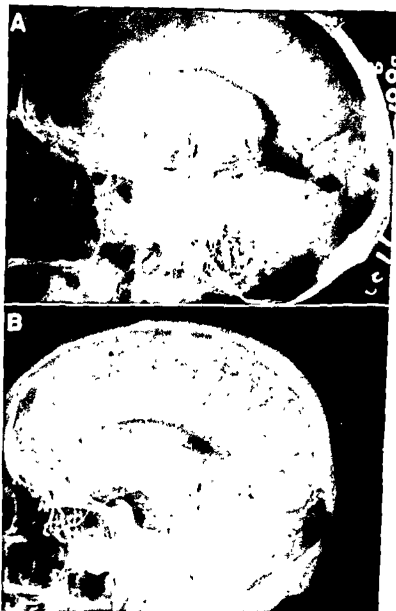


FIG 15 —Cryptococcal meningitis simulating neoplasm of the brain stem The ventriculoencephalogram, *A*, shows an obtuse angle of the aqueduct of Sylvius and poor filling of the cisterna pontis and interpeduncularis A rounded mass can be seen (arrow) just behind the posterior clinoids Normal ventriculoencephalogram is illustrated for comparison in *B* (Courtesy of Dr J Taveras, Department of Radiology, Neurological Institute, New York)

ocytosis and a lowered sugar or chloride value should stimulate search for an infectious agent The shorter clinical course of tuberculous meningitis, the greater frequency of the disease in children than in adults, the presence of a *proved* tuberculous focus in the lungs or lymph nodes, and the demonstration of causative organisms in the spinal fluid by direct examination or culture serve to differentiate the two diseases.

ulcers of the face which exuded glairy sticky pus, and which appeared before the "lesion" of the knee At first they were acneform and pointed. Later their apices became necrotic and the lesions formed small coalescing ulcers, which contained tenacious brownish red pus. Identical yeast-like organisms were isolated from this material and from the knee. Buschke reported spontaneous healing of some of the facial lesions in this case and even produced a similar eruption by injecting the organism into the patient's skin Rappaport and Kaplan's[174] patient with fatal cryptococcal meningitis also had an acneform lesion on the forehead 26 days following onset of illness It was a pointed indurated papule which became encrusted and appeared as an escharotic, reddened ulcer at autopsy. Histopathologic examination of the skin revealed only



FIG 18 —Surgical specimen resected subcutaneous cryptococcal granuloma of arm The shiny mucoid appearance of the cut surface resembles that of a myxomatous neoplasm From same patient illustrated in Fig 17 (Courtesy of Dr F Linell, Lund, Sweden)

a few cryptococci in the deepest layers of the epidermis and a heavy infiltration of the corium indicating the systemic nature of the disease

Linell, Magnusson and Nordén,[248, 249] reporting on the first case of cryptococcosis observed in Sweden, described cutaneous and subcutaneous lesions. Their patient, a 57 year old man with respiratory complaints, had pulmonary *infiltration of 4 years' duration presumed to be due to sarcoidosis Subsequently* he complained of headaches, and a number of pustular and acneform lesions appeared over the face and neck Biopsy and culture established the diagnosis of cryptococcosis Several of the facial lesions grew rapidly to form large strawberry-like masses (Fig 17) A subcutaneous tumor the size of a walnut and resembling a myxomatous neoplasm on cut section (Fig. 18) was then excised from the arm Nine months after the appearance of cutaneous manifestations and more than 5 years after onset of initial symptoms the patient died Autopsy revealed widespread dissemination of the fungus

lesion can be produced by diffuse cryptococcic meningitis with negative spinal fluid and in the absence of granuloma[60]. One example of a localized cryptococcal mass appearing as an expanding intracranial lesion is illustrated in the ventriculograms of Figure 15. These were made by Hochstetter[189] at the Neurological Institute, New York, in the case of a 59-year-old white woman who had chronic headache, fatigue, nystagmus, ataxia and other abnormal neurological findings due to chronic cryptococcal meningitis. Elevation of the floor of the third ventricle and poor filling of the cisterna pontis and interpeduncularis in the ventriculo-pneumoencephalogram were interpreted as evidence of an extra-axial mass ventral to the pons. The patient was not treated surgically, but received Actidione intrathecally and intramuscularly with good results. She remained asymptomatic 4 years after her discharge from the hospital in 1951.

3. INVOLVEMENT OF SKIN, MUCOUS MEMBRANES AND ADJACENT STRUCTURES

It is of interest to note that the first human case of cryptococcosis reported by Busse[50-52] is also the first recorded example of cryptococcal infection of the skin. The cutaneous lesions of that case are described[49,51,342] as small circular



Fig. 17.—Cryptococcosis of skin. Multiple lesions of varying size are present over the face and neck. The smaller lesions (arrows) are acneform nodules and pustules, while the larger growths actually resemble strawberries in size and general appearance. From the first recorded case of cryptococcosis in Sweden, published by Linell et al.[248,249] (See also Figs. 18 and 65) (Courtesy of Dr F. Linell, Department of Pathology, Lund, Sweden. AFIP Accession No. 701626.)



FIG 20—Widespread facial lesions resembling smallpox in a 7-year-old boy who had massive lymphadenopathy, abdominal enlargement due to hepatosplenomegaly, general debility, profound anemia, and lymphocyte counts of 70 and 78 per cent. The child died after ten days of hospitalization (about 3 months after onset of his illness). Autopsy revealed widely disseminated cryptococcal infection. Case reported by Soysal, Unat, and Tahsinoğlu.[**] (Courtesy of Dr Unat, Istanbul University, Istanbul, Turkey.)

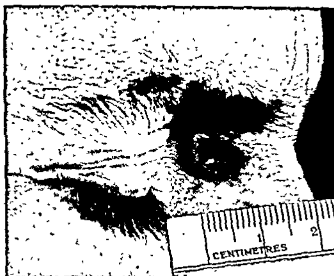


FIG. 21—Ulcerative cryptococcal granuloma which was thought to be a rodent ulcer. The lesion developed after the patient scratched himself. Subsequently, he had series of convulsive seizures and died. Autopsy revealed a large space-occupying cryptococcal granuloma of the brain (see Fig 16). (Courtesy of Prof W St C Symmers, London, England.[**])





Fig 19.—Extensive cutaneous cryptococcosis in a 31-year-old woman with granulocytic leukemia. In addition to the broad areas of involvement about the right eye, left nostril, and lips, there are acneform eruptions of the forehead and left eyelid (arrows). Discrete papules were also present over the upper extremities. Case 1 reported by Cawley et al.[illegible] (Courtesy of Dr. E. P. Cawley, Department of Dermatology, University of Michigan.) (See also Plate IA.)



FIG 20—Widespread facial lesions resembling smallpox in a 7-year-old boy who had massive lymphadenopathy, abdominal enlargement due to hepatosplenomegaly, general debility, profound anemia, and lymphocyte counts of 70 and 78 per cent The child died after ten days of hospitalization (about 3 months after onset of his illness) Autopsy revealed widely disseminated cryptococcal infection Case reported by Soysal, Unat, and Tahsinoğlu [309] (Courtesy of Dr Unat, Istanbul University, Istanbul, Turkey)

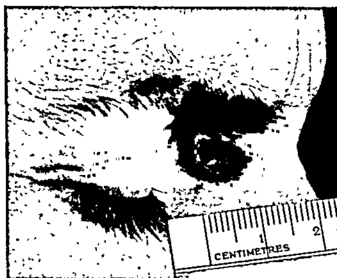


FIG 21.—Ulcerative cryptococcal granuloma which was thought to be a rodent ulcer The lesion developed after the patient scratched himself Subsequently, he had series of convulsive seizures and died Autopsy revealed a large space-occupying cryptococcal granuloma of the brain (see Fig 16) (Courtesy of Prof W St C Symmers, London, England [311])

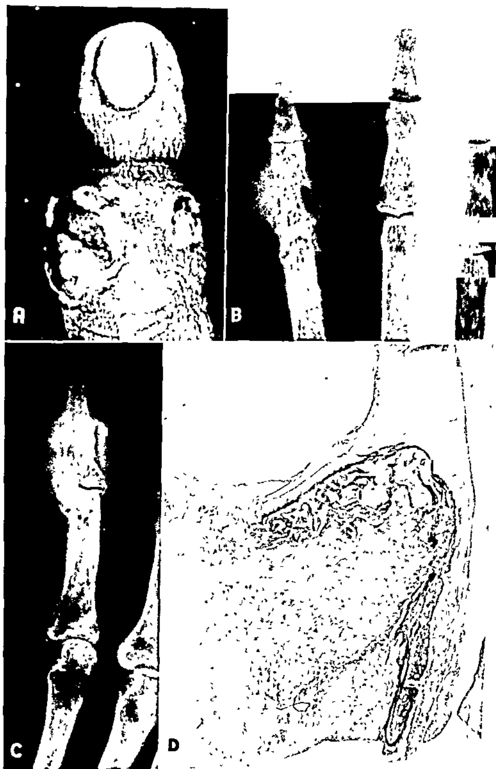


FIG 22—A Cryptococcal osteomyelitis, of the middle phalanx and proximal interphalangeal joint of a 59-year-old male Puerto Rican with no other evidence of infection The

The first review of cutaneous cryptococcosis was presented by Wile[449] whose patient with Hodgkin's disease had indurated lesions of the skin of the lower extremities. Sections through the skin revealed massive infiltration of the corium with cryptococci, lymphocytes, fibroblasts, and plasma cells, but no polymorphonuclear leukocytes. Organisms were free and also were contained within giant cells.

Relatively little attention has been given to the cutaneous manifestations of cryptococcosis, yet involvement of the skin and mucous membranes occurs more frequently than is usually suspected. Cawley, Grekin and Curtis,[66] in summarizing 120 cases of cryptococcosis, pointed out that 13 patients had cutaneous manifestations of the disease. It was noted that infection of the skin produced acneform lesions, papules, nodules, abscesses, ulcers, superficial granulomas, plaques resembling ecchymoses, and sinus tracts (Figs 17–21). One fatal case has been reported in which a diagnosis of measles was made because of a generalized morbilliform eruption, photophobia and headache at onset of the clinical disease.[12] Cutaneous lesions have involved the face, scalp, neck, trunk, and extremities. Of the various eruptions encountered, only those of acneform character appear with sufficient regularity to be of diagnostic significance. They are initially small, conical and non-tender (Fig 19), then enlarge slowly, ulcerate and coalesce with other lesions to involve broad areas. Several cases with severe facial disfigurement have been reported (Figs 17, 19, 20, and Plate IA). Lesions resembling furuncles were produced in a monkey by Weidman.[452]

Direct extension of cryptococcal infection from osseous lesions to overlying subcutaneous tissue and skin may occur[304] (Fig 22). Involvement of the mucous membranes may be the result of spread from a contiguous lesion or may occur independently. Mucosal infections have appeared as nodules, violaceous granulations, tumor masses and superficial ulcerations of the hard and soft palate, tonsillar pillars, tongue, gums, nasopharynx, and nasal septum.[66,432] Cryptococcus infection of mucous membrane may also cause formation of a tenacious, mucoid membranous exudate.[306] The current literature indicates that the mucous membranes are affected only about one-third as often as is the skin, while in earlier reports[146,147] a reversed relationship was noted.[453] E. L. Jones[206] reported a case in which there was extensive cryptococcal infection of the nasopharynx in a patient whose chief complaints included deafness and tinnitus. Treatment consisted of repeated excision of mucosal nodules followed by

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lesion developed after a box fell on the finger. One week after injury, x-rays (not shown here) revealed a poorly defined cyst-like structure in middle phalanx, broadening of the shaft and *thinning of the* cortex, and a *faint* linear *fracture* of the lateral cyst wall. These observations suggested a fracture through a chondroma. Patient returned seven and one-half weeks later, at that time he stated the finger had "broken open" and drained dirty, foul material. *B* and *C*. X-rays revealed progression of the destructive process with involvement of the proximal interphalangeal joint, all consistent with osteomyelitis. Amputation was performed through the proximal phalanx. *D*. Microscopic examination revealed a granulomatous osteomyelitis with extension into joint and through bony cortex to the skin. (See also Fig 46.) (Case contributed by Dr. W. B. Crawford, S. A. Surgeon, USPHS and Dr. H. H. Leffler, formerly Pathologist, Providence Hospital, Washington, D. C. AFIP Accession No. 606534.)

cauterization of their bases, potassium iodide, and x-irradiation. Two years after the patient was first seen, the infection was considered arrested.

Cutaneous and mucous membrane involvement in man is usually a manifestation of disseminated infection. Seldom has there been convincing evidence that a particular lesion represented a primary infection. Johns and Attaway[202] however, reported a small superficial cryptococcal granuloma that developed at the angle of the jaw after injury by a razor cut. The patient later died of meningitis caused by the same organism. Although an autopsy was not obtained, this was thought to be an example of a primary lesion. Berghausen[29] recorded an instance of cryptoccal infection developing at the site of injury to the tongue caused by a piece of hot steel.

While the data in animals suggest that inoculation is an important means of entry (see Cryptococcosis in Animals, page 39) cutaneous lesions developing in man coincident with trauma should not necessarily be interpreted as evidence that the fungus was introduced exogenously by inoculation. Symmers'[414] first patient had a painless facial lesion situated anterior to the right ear "at the site of an abrasion while shaving" and another on the nose "where he had accidentally scratched himself with a finger-nail." Both appeared as typical rodent ulcers (Fig 21). Excision and histopathologic examination of the preauricular lesion revealed it to be a cryptococcal granuloma. Meanwhile a third lesion appeared at the site of a razor-cut on the chin. Although cultures of the spinal fluid, blood and sternal marrow remained sterile, *C neoformans* was recovered from the cutaneous ulcers, sputum and urine. The evaluation of the patient's history in a case of this sort may vary. It seems unlikely that exogenous infection should be introduced on three separate occasions from soil, milk, fruit or other sources. Since the sputum did contain organisms, it is possible that the patient may have reinoculated himself. It seems less plausible to suggest that the cryptococci lodged in the skin hematogenously *after* trauma since healing of the surgical wound occurred without complication after excision of the preauricular lesion. However, cutaneous or subcutaneous involvement has occurred following surgical incision of deep-seated lesions.[313] The most likely explanation is that the lesions spread hematogenously to the skin and remained unnoticed by the patient until the time of minor trauma.

4 INVOLVEMENT OF BONES AND JOINTS

The first case of osseous cryptococcosis was the famous Busse-Buschke case. The patient was a 31-year-old woman with an infection of the tibia which spread to the knee and subsequently involved the lungs, spleen and kidney. Since that time bone manifestations have been reported in approximately 10 per cent of the cases, less than that which occurs in North American blastomycosis, coccidioidomycosis and actinomycosis. Collins,[76] who reviewed 200 cases of cryptococcosis, found 17 instances of bone involvement and added 3 new cases.

In reviewing case reports of osseous cryptococcosis, one notes the association of both pain and swelling with the bony lesions. For example, the patient of Smith and Crawford[390] had pain in the left shoulder and arm for 1 year, as well as a diffuse, smooth swelling over the scapula. Biopsy of this bone suggested Hodgkin's disease and x-rays suggested osteogenic sarcoma. A review of

the sections after autopsy showed a few scattered and degenerated cryptococci in fibrous tissue. Mider, Smith and Bray[258] reported a patient with an indurated painful swelling over the right sternoclavicular joint which produced a thick sanguinous fluid when incised. At autopsy, the surfaces of the joint were found to be eroded. In Jesse's[261] patient the initial symptom was pain in the right hip,



FIG 23—A X-ray showing localized area of bone destruction in proximal end of humerus with sharp scalloped margins and without bone reaction B, X-rays sixteen months later, the lesion has enlarged slightly, the margins are less distinct, and there is no evidence of periosteal reaction This was a 27-year-old woman whose illness began with a profuse postpartum vaginal discharge Over a period of two years the patient had numerous hospital admissions because of septic endometritis, phlebitis, and osteomyelitis. Involved bones included the humerus, cranium, vertebrae and pelvis (Fig 26). Virulent *C neoformans* was cultured from a lesion in the right ilium and from an abscess of the scalp The patient died with signs of basilar meningitis, and autopsy confirmed the diagnosis of generalized cryptococcosis Case 1 reported by Collins." (AFIP Accession No 537918, contributed by Dr M N Richter, Professor of Pathology, New York University School of Medicine X-rays obtained through courtesy of Dr R Wigh, Department of Radiology, Presbyterian Hospital, New York City.)

which awakened him at night. X-rays revealed a cyst 2 cm in diameter in the inferior ramus of the right pubis The cyst was removed surgically by resecting the major portion of the right inferior pubic ramus and the patient was well 1 year later In Collins'[76] Case 1 there was pain in the shoulders, hands and legs and tenderness over the right humerus, right iliac crest and pubic bones X-rays revealed multiple areas of bone destruction in the humerus (see Figure 23), skull, 3rd lumbar vertebra, right iliac wing and both ischial tuberosities. Chest

x-ray revealed widespread peribronchial infiltration (Fig. 8). Pain also was a symptom in Collins' Case 2, in this instance in the right shoulder and hip. X-rays showed well defined areas of bone destruction in the right scapular spine and in the alae of both iliac bones

Isolated lesions of bone, not associated with disseminated infection, are very *unusual* (*Fig 22*). *However*, it is in such cases that clinical diagnosis is most important and difficult, since these isolated lesions are likely to be considered

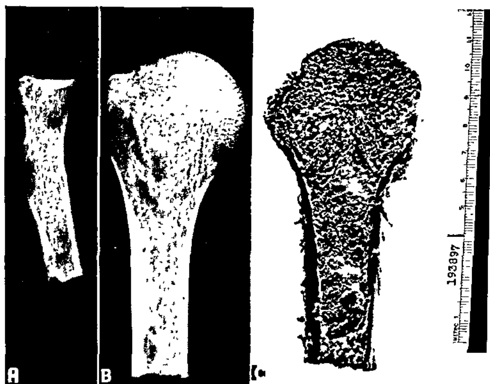


Fig 24 —X rays of osteolytic lesions of, *A*, proximal ends of radius and, *B*, humerus in a 53-year-old white man who also had osseous lesions of the femur and ilium The patient died of cryptococcal meningoencephalitis after a chronic relapsing febrile illness of 10 years duration C Section of the proximal humerus revealed rounded masses of mucoid inflammatory *exudate within the marrow cavity* Case *reported by* Wiener[460] (AFIP *Accession No* 193897, contributed by Veterans Administration Hospital, Coral Gables, Florida)

malignant neoplasms[91,177,190] until the correct diagnosis is made by recovery of the organism

Most bone infections represent hematogenous dissemination of the organism and are associated with widely distributed, multiple, discrete lesions (Figs 23, 24 and 26) Although practically every bone has been included in the reported cases of osseous cryptococcosis, there seems to be a predilection for bony prominences[76] (Fig 25), cranial bones and vertebrae Joint lesions are extremely rare and, as in other systemic mycoses, are associated with involvement of the adjacent osseous structures (Fig. 22) In other cases, the bone is involved by direct extension from a primary lesion located in the adjacent soft

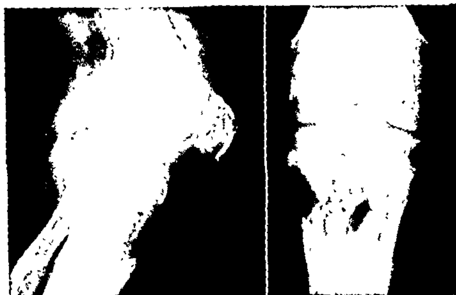


FIG. 25.—Osteolytic lesion with sharply scalloped margins in proximal end of tibia. There is complete destruction of tibial tubercle. (Courtesy of Dr. R. Wigh, Department of Radiology, Presbyterian Hospital, New York City.)

FIG. 26.—A. Symmetrical osteolytic lesions of the ischii, from the same case as illustrated in Fig. 23. The x-ray taken 14 months later reveals marked diminution in size of bilateral lesions and no increase in density of adjacent bone. The inguinal soft tissue mass (arrows) represents a psoas abscess which drained three days after x-ray, yielding 750 ml. thick pus.

tissues, sinuses, middle ear or meninges. Localized lesions of a single bone, amenable to surgical treatment, are extremely unusual but a few "cures" have been reported.[76] Rigdon and Kirksey[313] reported the most unusual case of a 31-year-old white man in whom spread of cryptococcal infection from the lumbar vertebrae produced a mycotic aneurysm of the abdominal aorta.

Although x-rays of osseous cryptococcosis available for study are too small in number to establish a characteristic x-ray appearance, they closely resemble those of North American blastomycosis and coccidioidomycosis and differ in many respects from actinomycosis.[76] Lesions of osseous cryptococcosis, in common with those of North American blastomycosis and coccidioidomycosis, are usually multiple, widely disseminated and discrete. They tend to be destructive, chronic and slowly changing. Radiologic progression is apparent in the slow enlargement of an area of bone destruction (Fig. 23), and regression may occur with slow reformation of normal bone (Fig. 26). The relatively stationary roentgenographic appearance of the bone lesions should suggest the possibility of cryptococcal infection.[76] These osseous manifestations are also seen in North American blastomycosis and coccidioidomycosis. In these mycoses, lesions are disseminated and discrete, in actinomycosis they are localized, extending by direct continuity to riddle adjacent bones. Periosteal proliferation is not characteristic of cryptococcosis while it is common in actinomycosis[263] and is also observed in North American blastomycosis[323] and coccidioidomycosis.[57]

5. INVOLVEMENT OF OTHER ORGANS

Any tissue of the body may be involved in disseminated infections, and occasionally one organ or tissue may appear to be affected selectively. However, in most disseminated infections, clinical and macroscopic evidence of lesions is lacking in sites such as kidney, adrenal, liver, spleen and lymph nodes. Infection of these tissues is discovered only on microscopic examination. In the most widely disseminated forms of the disease the heart, testis, prostate, and eye, in fact, any tissue of the body may be affected. With such extensive involvement the diagnostic potentialities of blood and urine culture, marrow aspiration and liver biopsy are apparent, yet seldom is the widespread dissemination discovered prior to autopsy.

In a few cases the clinical picture is dominated by symptoms related to a single organ. Massive involvement of the adrenal may produce the signs and symptoms of Addison's disease[112, 343] (Plate IC). Granulomatous lesions in the gastro-intestinal or genito-urinary tract may simulate tuberculosis or cancer.[169,439] In all such localizations the infection is presumed to be blood-borne from the lungs.

Eye involvement may occur in one of several ways. The most frequent is by direct extension of the infectious process along the subarachnoid space into the optic nerve (Fig. 13). In association with disseminated infection there may be multiple lesions in the uveal tract, retina, and vitreous[178, 442] (Fig. 27). Only rarely is cryptococcosis the cause of blindness in a patient who has no other evidence of cryptococcal infection (Plate IB). However, two such cases have been reported, both in septuagenary women, one of whom remained well

for 8 years after the onset[13] (Plate IB and Fig 66). Deep cryptococcal keratitis was reported by Fazakas[121] The eyelids and orbit may be involved by extension of contiguous skin lesions[66,95,118,303] (Plate IA)

[illegible] r system was reported by Long- [illegible] who died of cerebral cryptococ- [illegible] the outer part of the media of the thoracic aorta by *C. neoformans*. Although the elastic fibers of the media were spread apart and broken, cellular response was negligible. Rigdon and Kirksey[311] reported an aneurysm of the abdominal aorta, caused by cryptococcal

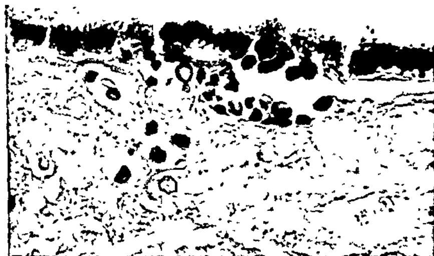


Fig 27.—Proliferation of cryptococci in choriocapillaris, posterior uveal tract of eye, in a case of disseminated cryptococcosis complicating reticulum-cell sarcoma Bauer stain × 900 (AFIP Accession No 262406, contributed by Army and Navy General Hospital, Hot Springs, Arkansas)

infection, in which hemorrhage from the aneurysm was responsible for the patient's death The initial and continuing complaint was severe back pain radiating down one thigh Cryptococcal endocarditis lenta may occur (see page 151).

6 ASSOCIATION WITH LYMPHOMA AND LEUKEMIA

[illegible]

other types of neoplastic disease[476] Some have suggested that the fungus or one of its products may be one of several etiologic agents of Hodgkin's disease[47,83] Others have entertained the idea that the cryptococcus may have evoked a histopathologic reaction simulating Hodgkin's disease[47,77,83,125,313] Zimmerman and Rappaport,[476] who studied 60 cases of cryptococcosis, found that 30 per cent occurred in patients with Hodgkin's or related diseases and regarded the evidence to be insufficient to support either of these hypotheses

Patients with malignant lymphoma or leukemia are known to be more susceptible to infectious diseases, one of which happens to be cryptococcosis. In fact, these unfortunate individuals may be infected simultaneously by several different infectious agents.[352,476] There is a possibility, therefore, that in such cases the pathogenesis might be different from that of other cryptococcal infections which are thought usually to begin in the lungs. It is entirely possible that in the patient with a malignant disease of the reticuloendothelial system, ordinarily feebly pathogenic cryptococci present on the skin, in the lungs or gastrointestinal tract may become invasive as a result of altered host-parasite relationships incident to disturbed physiology of the reticuloendothelial system. The alteration of lymphatic tissues in Hodgkin's disease with concomitant depression of the body's immunologic response may permit cryptococci or other adventitious organisms to gain a foothold and produce disseminated disease. Some support is given to this concept by the widespread dissemination of cryptococci during the course of Hodgkin's disease, lymphosarcoma, and leukemia. Such dissemination is unusual in the ordinary case of cryptococcal meningoencephalitis.

It is of interest to recall that the first human case of cryptococcosis, that reported by Busse,[50] was in a 31-year-old woman who for years had suffered from considerable lymphadenopathy. This suggests that association of this mycosis and malignant diseases of the reticuloendothelial system may have existed since the first reports of cryptococcosis, though the relationship has been emphasized only recently.

7. RELATION TO SARCOIDOSIS

In localized forms of cryptococcosis, particularly in the lungs and lymph nodes, the tissue response may mimic the non-caseating tubercles which are so characteristic of sarcoidosis In many of these cases, however, prolonged search aided by the use of special stains for cryptococci will reveal the causative fungus There are, on the other hand, well-documented cases in which sarcoidosis appeared to co-exist with cryptococcosis[77] We have studied cases of this type (Figs 10 and 44) and have made an intensive search for organisms in the apparently healing tuberculoid granulomas, but were unsuccessful in demonstrating the organisms in them, yet other tissues from the same patient revealed lesions containing a myriad of cryptococci

A good example of such cases was reported independently by Fisher[124] and by Collins[76] A 20-year-old colored woman gave a 2½ year history of fatigue, anemia, amenorrhea, loss of axillary and pubic hair, and ankle edema Lymph node biopsy led to a diagnosis of sarcoidosis Tibial pain developed with roentgenographic evidence of lesions in the tibia and ischium Cultures of a skin lesion taken at the time of tibial biopsy revealed the presence of cryptococci. A fluctuant mass formed over the ischium, and subsequently the patient died of cryptococcal meningitis At autopsy, lesions containing cryptococci were found in the nervous system, kidney, adrenal, lungs, bones, and subcutaneous tissues. Other lesions typical of sarcoidosis but not containing cryptococci were found in the lungs, heart, lymph nodes, liver, spleen and pituitary gland

Another case of osseous cryptococcosis associated with sarcoidosis of the

lymph nodes was described in a 22-year-old colored American soldier who had asthma of 8 months duration, malaise, anorexia, weight loss, and chest pain * Physical examination revealed generalized lymphadenopathy and chest x-ray disclosed a widened mediastinal shadow as well as bilateral infiltration of the lung fields Malignant lymphoma was suspected, but excision and pathologic examination of an epitrochlear lymph node led to a diagnosis of Boeck's sarcoid. Needle biopsy of an osteolytic lesion of the right iliac crest yielded only degenerated bone. The patient received cortisone orally and showed symptomatic improvement and regression of lymphadenopathy Upon arrival at the 1100th Air Force Hospital, Washington, D.C, he was febrile and complained of low back pain which radiated to the left knee. Substitution of ACTH for cortisone therapy resulted in a recurrence of previous symptomatology, necessitating resumption of cortisone. A tender swelling appeared over the left hip, and approximately 250 ml of thick, brownish pus was evacuated from beneath the left gluteus maximus muscle. Culture studies of this exudate and pathologic examination of the excised tissue established the infection as cryptococcosis Treatment with nystatin (Mycostatin) by parenteral and oral routes for more than three months was followed by healing of the hip lesions A repeat epitrochlear lymph node biopsy at this time still showed conglomerate epithelioid tubercles and no fungi were demonstrable by culture or special staining technics Three months after termination of this therapy an osteolytic lesion appeared at the distal end of the right radius The patient showed no manifestations of cerebral cryptococcosis for 18 months after hospitalization, up to the present time (see pages 139 and 148 for nystatin therapy, and page 150 for effect of hormones).

The potential danger to the patient that accompanies cortisone therapy of sarcoidosis is again illustrated in a similar case described to the authors by Professor Symmers of London Identical, male, 33-year-old twins, previously in excellent health, were found to have typical radiologic manifestations of miliary pulmonary sarcoidosis with hilar lymph node involvement Both had hyperglobulinemia and both were negative to 1 100 dilution Mantoux skin test reagent Lymph nodes and tonsils of both patients were sarcoidal in character and cryptococci could not be demonstrated One twin received a course of cortisone therapy, following which he developed a fluctuant abscess of the shoulder which, on x-ray, revealed considerable destructive osteitis *Cryptococcus neoformans* was recovered from the abscess material The symptoms in this patient subsided and he remained well, except for headaches which appeared at the time this case history was received It is of interest to note that, in all 3 of the cases cited, sarcoidosis of the lungs and lymph nodes was associated with active osseous cryptococcosis

How may such cases be interpreted? Since the occurrence of epithelioid tubercles is not specific and may be associated with such diverse pathological processes as tuberculosis, histoplasmosis, syphilis, berylliosis, and cancer, we choose to believe that when seen in association with disseminated cryptococcosis, it represents a reaction pattern at a site where the host tissues have successfully coped with the fungus. It seems unlikely that cryptococcus infection can be responsible for more than a very occasional case of sarcoidosis

* Heller, S, McLean, R A, Campbell, C C and Jones, I H A case of coexistent nonmeningitic cryptococcosis and Boeck s sarcoid (To be published)

VI. Cryptococcosis in Animals

SOME OF THE earliest publications on the cryptococcus and the diseases produced by it concerned strains of animal and plant origin. For example, in 1901 Klein[213] described a pathogenic yeast he recovered from milk, and the following year Weis[455] reported that this organism was identical with strains of human and plant origin. Barron,[18] who recently reviewed the world literature on spontaneous cryptococcosis of animals, found references to the isolation of a cryptococcus from the lymph node of an ox by Sanfelice[339] in 1895, and to Vuillemin's



FIG 28.—Ulcerative granuloma of palate in a male tiger cat. *The pale areas in the subepithelial tissues are heavily laden with thickly encapsulated cryptococci.* Fungus cells are also observed on the surface of the palatine mucosa. × 75. Case reported by Holzworth.[185] (AFIP Accession No 324207.)

demonstration of pulmonary cryptococcosis in a pig in 1901. Since these early reports, spontaneous cryptococcosis has been observed in the horse,[139,211,293,364,452] dog,[190,270,308,374] fox,[120] cat,[195,196,270] cheetah,[162,454] civet,[120] monkey,[120,419] guinea pig,[90] ferret,[398] and in dairy cattle.[108,199,235,274,324,356] The fungus has also been recovered from pigeon droppings,[109,110] but it is of interest to recall that spontaneous infections of birds has not been reported to date, possibly because they are protected by their high body temperature (Table VII, page 127).

Dr W. H. Eyestone has had personal experience with 3 cases at the Washington Zoo during a period of 2 years. His first case was one of systemic cryptococcosis with nervous system involvement in a small-toothed palm civet. The second case, occurring in an arctic fox, was also proved at autopsy. The brain and a mesenteric node were found to be infected. Eyestone's most interesting case was that of a sooty mangabey with an enlarged axillary lymph node The animal had been imported from South Africa only 3 months before and was thought to have a lymphoma. Pathologic studies failed to confirm this clinical impression but demonstrated instead that the nodal enlargement was due to cryptococcosis. The monkey remained well for over a year.

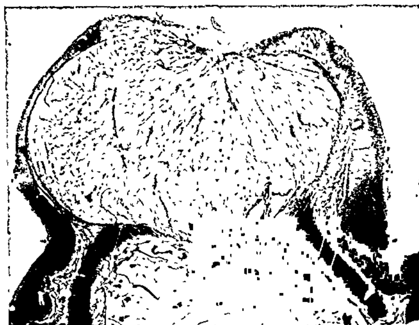


FIG 29—Massive growth of cryptococci which formed a firm nodular mass in the stomach wall of a cat Although the proliferation of organisms has occurred predominantly in the submucosa and muscularis, there is also involvement and ulceration of the gastric mucosa × 7. (AFIP Accession No 523653, contributed by Dr John Mills, University of Pennsylvania School of Veterinary Medicine) (See also Fig 30)

Study of natural infections in animals may throw some light on the pathogenesis of cryptococcosis in man In a number of these cases, particularly in the horse, cat and dog, prominent lesions were noted in the facial region, especially about the nose and palate (Fig. 28) These locations suggest that the infection might have been acquired by direct inoculation of the fungus, which is free in nature In other instances there was no obvious external lesion, but extensive pulmonary involvement, indicating that the infection might also have been air-borne In still other cases where no facial or pulmonary lesions exist, the demonstration of a massive granuloma in the stomach or intestines suggests the alimentary tract as a portal of entry (Fig 29) In common with fatal in-

fections of man, those in animals usually are also due to nervous system involvement[15,270] (Fig 30). Histopathologic reactions in animal infections do not differ materially from those in man (Figs. 30 and 31) (see Pathology, page 53

Outbreaks of mastitis in dairy herds are of paramount importance epidemiologically as well as economically. It is recalled that in England, Carter and Young[56] isolated *C. neoformans* from milk produced by apparently normal cows In the Maryland enzootic[324] 106 cases appeared during the



Fig 30 —Extensive cryptococcal meningoencephalitis in a cat The subarachnoid space, especially on the ventral surface of the brain, is extremely thickened by an accumulation of fungus cells and their mucinous capsular material The process extends along the sulci which are markedly widened Cystoid accumulations are also deep in the cerebral parenchyma (arrows) × 4 Same case as Fig 29 Compare with similar process in human cryptococcosis, Fig 39 *The cat had been observed to be listless and ataxic It would stand with front legs spread apart but showed a tendency to fall to the right* Occasionally it would lie on its right side with right legs flexed and left legs extended (AFIP Accession No 523653)

course of a year among 235 head of Holstein-Friesian dairy cattle The infection oculation of the fungus into the teats, probably ines One or more quarters of the udder were to regional lymph nodes and, at times, to the external iliac or deep inguinal nodes was observed (Fig 33) Evidence of hematogenous spread was found in only one animal with pulmonary involvement (Fig. 34) Infection of the nervous system did not occur The responsible fungus was shown by Emmons[104] to be typical *Cryptococcus neoformans*, pathogenic for mice, but apparently of limited virulence for cattle A 10-week-old calf was exposed in various ways by feeding 200 ml. of broth culture in milk, by intravenous injection of 5 ml of culture, and by subcutaneous inocula-

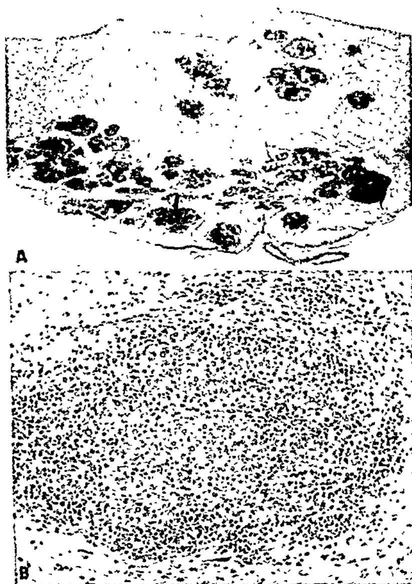


Fig 31.—Granulomatous cryptococcal encephalitis in a 4-year-old cocker spaniel. There is a remarkably intense tissue reaction and relative paucity of fungus cells in this case. A Pontine lesions, × 8. B Same, × 160. (AFIP Accession No 505161, contributed by Dr C F Helmboldt, University of Connecticut Department of Animal Diseases.[256])

tion of 0.5 ml. of culture into the cervical region. Thirty-seven days later it was *impossible to demonstrate any organisms or lesions in the tissues*.[324]

Clinical manifestations in the cows were of gradually increasing severity and included abnormal firmness and fullness of the mammary glands (Fig 32), marked reduction in milk flow, and change to viscid secretions. The slight

elevation of temperature and the absence of toxemia served to differentiate the cryptococcal from the usual forms of mastitis One of the most noteworthy characteristics of the mastitis observed by Pounden and co-workers[324] was the change in the character of udder secretions to a gray-white material so viscid that it would hardly flow down the sides of inverted sample bottles (Fig 35). The ducts in the udder were obstructed to such an extent that it was difficult to inject any appreciable quantity of solutions for treatment.

In the Wisconsin outbreak of cryptococcal mastitis, described by Simon and co-workers,[396] approximately 50 of 280 lactating animals manifested transient anorexia, fever, and diminished lactation 4 to 8 weeks following parturition. It

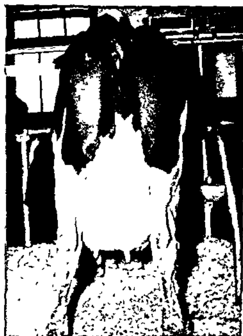


Fig 32 – Cryptococcal mastitis causing pronounced distention of the udder and extreme discomfort to the animal. The hind legs are spread apart because of the swelling From Pounden et al[324] (Courtesy Dr W. D Pounden, Agriculture Experiment Station, Wooster, Ohio)

had been a routine procedure in the management of this herd to infuse all quarters of each mammary gland with a penicillin mixture at the close of the milking period A glucose solution used for mixing the penicillin subsequently was found to be contaminated with pathogenic *C neoformans* Since this antibiotic does not interfere with the growth of cryptococci, the contaminated penicillin may have been responsible for this outbreak of bovine cryptococcal mastitis. In light of the recovery of *C neoformans* from the soil, it seems more likely that the original source of the organism was soil contamination of milking equipment and utensils.

The economic loss sustained in an outbreak of cryptococcal mastitis can be great Some cows in the Maryland enzootic had been producing 60 pounds of milk a day prior to the outbreak, but output was reduced to less than a third of this within a month and the cows were completely dry by the end of the second month Since no form of treatment was found satisfactory, control was based on such generally recommended procedures as detection and segregation

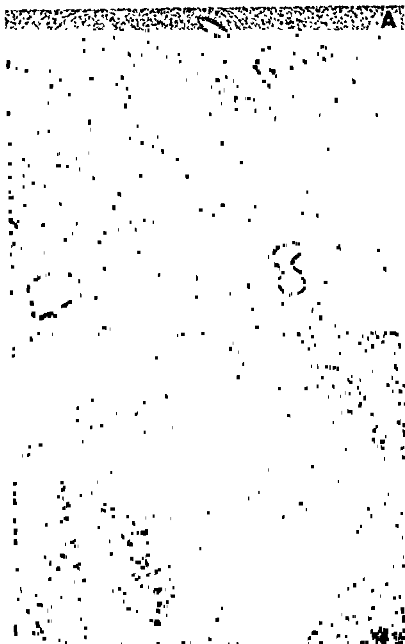


FIG 33 – Cryptococcal mastitis with spread to regional lymph nodes A Granuloma containing fungus cells, atrophy of mammary gland × 90 B Granulomas containing cryptococci in lymph node × 75 (AFIP Accession No 334620, contributed by Cdr W W. Ayres, U S Naval Medical School, Bethesda, Maryland)



FIG 34.—Cryptococcosis of bovine lung secondary to cryptococcal mastitis *A* × 95, *B* × 210 From the material reported by Innes et al [194] (AFIP Accession No 495648, contributed by Dr H R Seibold, School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Alabama)

of new cases from healthy stock, use of sanitary milking methods, correction of machine operation, sterilization of equipment, avoidance of transmission of infection by dairy personnel, and improvement of diet

The reward for this tedious and expensive program was the virtual elimination of infection within a year in those herds where the recommended control procedures were adequately applied The incidence of mastitis was not reduced in other herds in which the herdsmen failed to carry out these measures.

Experience with cryptococcosis in animals reinforces our belief that infection in man and animals is usually exogenous, probably soilborne, and that there



Fig 35—The abnormal udder secretion, characteristic of bovine cryptococcosis, is so viscid that it barely flows down the inverted tube From Pounden et al.[364] (Courtesy Dr W. D Pounden)

is no hazard of man-to-man or animal-to-man transmission, for even in outbreaks of bovine mastitis there were no reports of infection in herdsmen or in milk consumers Standard pasteurization methods effectively destroy the fungus (See Table I, page 46)

A chronic infectious disease of the skin, subcutaneous tissue, mucous membranes and regional lymphatics of horses, mules and donkeys, often called epizootic lymphangitis, has been ascribed to the fungus *Cryptococcus farcinimosus*[25,46,340,429] *In tissue this causative* organism has a striking resemblance to *Histoplasma capsulatum*[362] and it produces spores and hyphae on culture. Because of its morphologic similarity to *H capsulatum*, Ciferri, Redaelli and Visocchi[71] placed the organism in the genus *Histoplasma* as *H farcinimosum* Although the exact taxonomic position of the etiologic agent of epizootic lymphan-

gitis is still under debate,[71,92,100] there is no longer any reason to consider the organism a member of the genus *Cryptococcus.*

TABLE I.—SUSCEPTIBILITY OF *C. NEOFORMANS* TO HEAT

Temperature		Period of Exposure	Result	Reference
C.	F			
40 6°	105 1°	24 hrs	Most of cells dead	Kuhn[224,226]
42°	107 6°	30-50 hrs	Death	Benham[25]
50°	122°	42 min	Death	Crone, de Groat and Wahlin[64]
55°	131°	5 min	Death	Emmons[108]
60°	140°	5 min	Death	Crone, de Groat and Wahlin[64]
60°	140°	<5 min	Death	Cox and Tolhurst[63]
62 8°	145°	30 min *	Death	Emmons[108]
73 9°	165°	1 min †	Death	Pounden et al.[274]

* Pasteurization process (holding).

† Flash pasteurization

VII. Immunology

Although pathogenic fungi are considered to be more allergenic than bacteria because of the pronounced skin hypersensitivity of patients with certain of the mycoses, they are less active in producing detectable circulating antibodies Thus low antibody titers which ordinarily would be ignored in other microbial infections assume greater importance. It is likely that unavailability of perfected fungal antigens and incomplete development of the immunology of my- [illegible] the true antigenicity of

[illegible] een that C neoformans is poorly antigenic upon injection into animals. Nevertheless, moderately high agglutinin titers to this organism have been obtained in animals by Benham[23] (1:160), Hoff[191] (1:280) and Evans[113] (1 320). Benham,[23] in 1935, observed that treatment of the cryptococcus capsule with dilute acids enabled her to obtain even higher agglutinin titers, however, similar treatment with acid had no effect upon size or morphology of the capsule.[295] More recently Neill, Abrahams and Kapros[295] discovered that although strongly encapsulated strains of *C. neoformans* were practically non-antigenic, thinly encapsulated strains were fairly potent immunizing agents. The poor results obtained by previous investigators in production of immune serum were attributed to small and infrequent immunizing doses rather than to the poor antigenicity of the organism It is possible to produce adequate immune anticryptococcal rabbit serum with regularity by injecting 500 million thinly encapsulated cryptococci every other day for 7 injections to achieve agglutinin titers of 1 160 to 1 640[295]

1 ANTIGENIC AND CHEMICAL STRUCTURE OF THE CRYPTOCOCCUS

The genus *Cryptococcus*, comprising both saprophytic species and the pathogen, *C neoformans*, was divided by Benham[23] into four groups based upon serologic and morphologic differences Strains of *C. neoformans* recovered from human infections were placed by her in Group III Employing reciprocal agglutinin-absorption technique, Evans[113] further sub-divided this group into 3 serologic types A, B and C

The only antigen of the cryptococcus studied thus far has been its capsular polysaccharide Kligman[214] isolated the polysaccharide in the crude state and, because it failed to induce either precipitins or skin hypersensitivity in animals, concluded that it was non-antigenic for rabbits and mice Neill and coworkers,[296] using a purified polysaccharide, first obtained from the cryptococcus capsule by Hehre, Carlson a[illegible]
by its prompt precipitation [illegible]
corroborated by Evans and [illegible]
saccharide of the cryptococcus in precipitin absorption techniques with hyperimmune rabbit serum and again differentiated three antigenic types A, B and C

Drouhet, Segretain and Aubert[98] and *Evans and Mehl*[117] independently showed by means of filter paper partition chromatography that cryptococcal polysaccharide was composed of units of xylose, mannose and uronic acid, and probably glucuronic acid. Evans and Theriault[119] further separated the cryptococcal polysaccharide Type B by paper chromatographic partition into two fractions, SB_1 and SB_2, and identified hydrolysis products by paper chromatography as xylose, *mannose and glucuronic acid. Studies of the capsular polysaccharide* of non-virulent *C. neoformans* var. *innocuous* by Einbinder, Benham and Nelson[102] revealed the presence of 6.7% hexuronic acid, expressed as glucuronic acid, 18.1% hexose, expressed as glucose, and 31.0% pentose, expressed as arabinose. The capsular material contained no starch, glycogen, amino acids, protein, amino sugar, nucleic acid contaminants, hyaluronate or mucoitin sulfate. Drouhet and Segretain[97] reported destruction of the capsule by hyaluronidase, and assumed that it was composed partially of hyaluronic acid. However, Foley and Uzman[129] *treated cryptococcal polysaccharide solutions with hyaluronidase (Alidase,* Hydase) and failed to produce any measurable change in viscosity. Furthermore, incubation of viable, encapsulated cryptococci at 25° and 37°C in the presence of 1 to 300 T.R.U.* of hyaluronidase in acetate buffers at pH 4.5 and 6.0 or in phosphate buffers for intervals varying from 5 minutes to 24 hours failed to show evidence of decapsulation. Our own experiments with saline suspensions of viable cryptococci in the presence and absence of 150 T.R.U./ml hyaluronidase (Wydase) at 20° and 37°C and at pH 4.0 and 7.0, examined in 1 hour and 24 hours, failed similarly to reveal a decapsulating effect of hyaluronidase. Although the capsules were smaller at pH 4.0 than at 7.0, the presence or absence of hyaluronidase made no apparent difference in capsule thickness.

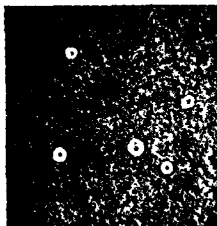
A general test for the presence of mucopolysaccharides, one of which is hyaluronic acid, is metachromatic staining with aqueous toluidine blue.[245] Histochemical studies of thickly encapsulated cryptococci in sections of human lung by Johnson and Gridley[205] revealed no change in metachromasia after treatment with hyaluronidase. Control sections of umbilical cord, on the other hand, showed complete absence of metachromasia following treatment with the same enzyme preparation. This is corroborative evidence for the absence of hyaluronic acid in the capsular substance of *C. neoformans*.

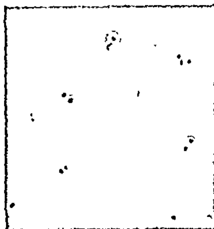
Presumed endotoxic substances of *C. neoformans* have been demonstrated to kill white Swiss mice within 48 hours after intraperitoneal injection of dead cryptococci combined with an adjuvant of 1 to 2 mg. dried tubercle bacilli to each dose.[356] LD_{50} for *C. neoformans* was 3.4 mg. of acetone dried cells.

2. SEROLOGY IN DIAGNOSIS

Agglutinins to *C. neoformans* and complement-fixing antibodies have seldom been demonstrated in the past in the blood stream of patients, although Rappaport and Kaplan[325] found agglutinins to this organism in titer of 1:40. More recently Neill, Sugg and McCauley,[295] by means of precipitin and complement fixation reactions employing specific hyperimmune anti-cryptococcal rabbit serum, found *soluble cryptococcal antigen to be present in the spinal fluid, blood*

* Turbidity reducing units





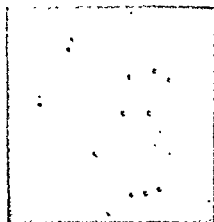


FIG. 36—*C. neoformans* (serotype B) A India-ink mount B Specific homologous rabbit antiserum type B C Heterologous rabbit antiserum Type A A capsular reaction occurs in the presence of homologous serum and is absent in heterologous serum The capsule, which is outlined by specific serum, is no greater in size than in India-ink mount, illustrating that capsular swelling does not occur in the "Quellung" reaction, thus supporting the use of the term "capsular reaction" From Evans and Harrell[115] (Courtesy Dr. E E Evans, University of Michigan)

and urine of a patient with cerebral cryptococcosis. This followed the demonstration of soluble cryptococcal antigen in the blood and tissue of cryptococcus-infected mice by means of precipitation of tissue extracts with homologous type hyperimmune rabbit serum.[297] The demonstration by Neill, Sugg and McCauley[298] of the presence of cryptococcal polysaccharide in the urine is of special interest to us since we have found in kidney sections renal tubular casts that, like the cryptococcal polysaccharide, were mucicarmine-positive and stained intensely by the Gridley technic[137] (Plate IIA). These casts were present in the kidneys of several patients who died of disseminated cryptococcosis.

It is now possible to type *C. neoformans* by means of the capsular reaction or by agglutination using type specific rabbit antiserum[113,114] (Fig. 36). Cross reactions may occur, however, with *Candida albicans, Saccharomyces cerevisiae,* trichophytin extract, type 2 pneumococcus and other antigens.[118]

Neill and associates[296] pointed out that "Quellung" or outlining of the cryptococcal capsule by a specific immune serum is not the swelling of an originally thin capsule, since India-ink preparation of the same organism with non-specific serum showed capsules of the same size. For this reason, we prefer the term "capsular reaction" to "Quellung" for *C. neoformans* (Fig. 36). Tomcsik and Guex-Holzer[430] studied the behavior of encapsulated microorganisms, including *C. neoformans,* towards proteins of different isoelectric points and observed that the cryptococcal capsule could be made visible by the addition of non-specific proteins. This occurred at a narrow pH range lying on the acid side of the isoelectric point of the protein tested and the optimal pH depended upon the capsular substance of the microorganism. This nonspecific capsular reaction took place through a salt-like combination of several proteins with the capsule of the organism and could be reversed by shifting of the pH.

In interpreting this phenomenon, one must bear in mind that physical and immunologic properties of polysaccharides are remarkably influenced by the presence of even low concentrations of bound protein or peptide residues in them, and that the isolation of polysaccharides in the soluble form from natural sources often involves drastic chemical purification methods.[400] Thus Einbinder and coworkers,[102] who made a chemical study of the polysaccharide of *C. neoformans* var. *innocuous,* used saturated KCl to prevent extraction of cryptococcal proteins and K_2CO_3 to aid in breaking up protein-polysaccharide complexes before proceeding with the analysis of the purified polysaccharide. Evans and associates[118,119] employed chloroform and *n*-butanol extraction method for the removal of proteins from capsular polysaccharide before making serological studies. Since Tomcsik and Guex-Holzer's[430] observations indicate that a *non-specific* capsule reaction is dependent on a salt-like combination of several proteins with the organism's capsule, *specific* capsular reactions may be affected similarly. An investigation of the immunologic role of the protein-polysaccharide complex of the capsule similar to those performed with pure polysaccharide components may produce fruitful results.

A relationship has been established between gamma globulin synthesis and the ability to resist infections, as exemplified by congenital agammaglobulinemia.[42] In probing for an immunologic explanation as to why *C. neoformans* produces cerebral disease in some patients and not in others, and why it dis-

seminates widely in lymphomatous disease, it might be well to recall that ordinarily both the gamma and alpha, fractions of human serum globulin increase sharply with acute febrile disease[117, 161, 260, 380, 427] Failure of this serologic response to occur in certain individuals might account for inability to cope with the fungus

In a patient with cryptococcal meningitis treated at Mount Sinai Hospital, New York, by Littman and Nathanson[254] electrophoretic studies of the patient's blood serum were made 1 week before death In spite of overwhelming cryptococcal infection in the patient there was only slight increase of both gamma and alpha, globulins (Table II). This is of special interest since the

TABLE II —ELECTROPHORETIC ANALYSIS OF HUMAN SERUM IN CRYPTOCOCCAL MENINGITIS*

Components	% of total serum proteins	Normal values†
Albumin	43.5	55.2
Alpha₁ globulin	5.7	5.3
Alpha₂ globulin	5.5	8.7
Beta globulin	30.3	13.4
Gamma globulin	15.0	11.0

* Littman and Nathanson,[254] determinations by P. Bossak
† Armstrong, Budka and Morrison [7]

patient had been receiving, concomitantly with Actidione, large (4.5×10^9 cells) intravenous doses of typhoid-paratyphoid vaccine daily for fever therapy. Neither the fatal cryptococcal infection nor the typhoid vaccine injections stimulated appreciable antibody response in this patient Therapeutic administration of gamma globulin failed to halt the disease or produce a beneficial effect, but it may have been administered too late

3 SKIN HYPERSENSITIVITY

Little is known at present of the allergenic state in cryptococcosis and of the value of skin testing as a diagnostic aid With the steady technical improvements in the preparation of cryptococcal antigens, skin hypersensitivity in cryptococcosis is the next logical subject of investigation

Relatively crude suspensions of cryptococci have been employed for skin testing in the past with negative results However, Berghausen[23] injected a boiled, aqueous extract of *C neoformans* subcutaneously in a patient with cryptococcosis and observed a pronounced local skin reaction Kessel and Holtzwart,[210] using a broth filtrate of *C neoformans* heated to 60°C for 2 hours, made intradermal injections into 2 patients with cerebral cryptococcosis Twelve hours later an area of swelling and erythema measuring 2 cm in diameter appeared, reached its maximum size in 24 hours, and persisted for 5 days Dienst[91] injected a patient with cryptococcosis intradermally with 0.1 ml saline suspension containing one billion killed cryptococci per ml and an erythematous area 2 cm. in diameter appeared in 24 hours and disappeared in 48 hours

Carton[58] skin tested a patient with a 1 per cent saline suspension of the patient's organisms heated to 100°C for 10 minutes, and with a hydrochloric acid-treated decapsulated heat-killed suspension Wheals were produced in the skin with both types of antigen. Leopold's[236] patient with osseous cryptococcosis had a strongly positive skin test, a wheal 4 5 × 5 0 cm developing within 48 hours. No agglutinins were found in the patient's serum Torulin concentrate,* not yet marketed but available for experimental use, is diluted 1:1000 with sterile saline and 0 1 ml is injected intradermally

* The Lilly Research Laboratories, Indianapolis, Indiana

VIII. Pathology

TISSUE REACTION to *Cryptococcus neoformans* varies considerably from case to case and from organ to organ, nevertheless certain generalizations concerning the pathologic process may be made. The inflammatory response is essentially cellular. Tissue macrophages are the principal cellular elements observed and frequently they are the only inflammatory cells present (Fig 37) At times there are many giant cells (Fig 38) and dense infiltration by lymphocytes and

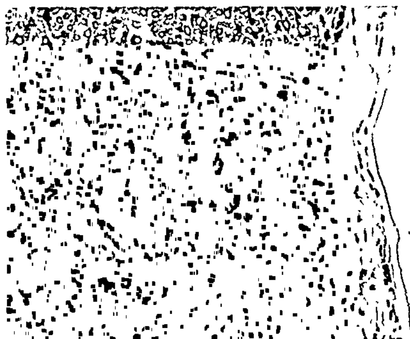


FIG 37—Subpleural cryptococcal granuloma Some of the fungus cells are free but most are contained within the cytoplasm of macrophages The pleura, seen on the right, shows minimal inflammatory reaction Mucicarmine with hematoxylin counterstain × 315 (AFIP Accession No 262100, contributed by Army and Navy General Hospital, Hot Springs, Arkansas)

plasma cells A significant humoral response is generally absent, hyperemia being slight and exudation of edema fluid, fibrin, blood, and polymorphonuclear leukocytes being minimal

Frequently the relative paucity of both cellular and humoral reactions is impressive, especially in the nervous system Great masses of cryptococci and an overabundance of their mucinous capsular material distend the meninges and

fill cystoid spaces within the parenchyma of brain and spinal cord (Fig. 39). On casual examination it may seem that within these lesions inflammatory cells are virtually absent. Scrutiny under greater magnification, however, will usually reveal many of the fungus cells within macrophages, the cytoplasm of which is so pale and distended by the mucinous material that they are barely visible (Fig. 40). Lesions of the thoracic and abdominal viscera, notably those removed surgically, are more often obviously granulomatous (Fig. 41) than are those of the brain or meninges. Nevertheless, any tissue may reveal lesions that

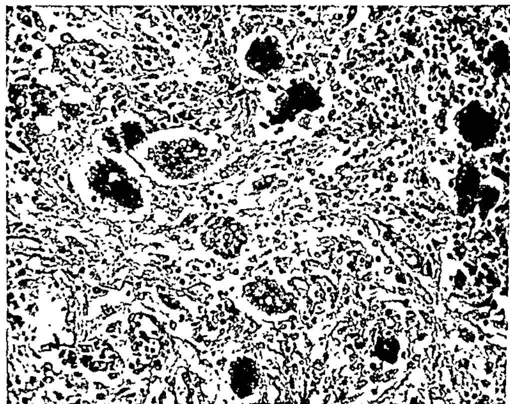


Fig 38.—Cryptococcal granuloma of lung Almost all the fungus cells are intracellular, many within giant cells Bauer stain × 370 (AFIP Negative No 218120-518)

appear to be essentially acellular or that are densely infiltrated with chronic inflammatory cells

Non-reactive lesions observed in various locations may be misinterpreted as myxomatous neoplasms (Figs 18 and 42), in the respiratory tract they may resemble mucous polyps [289] On a mucosal surface they may give rise to a tenacious mucoid membranous exudate [90] (Fig 19 and Plate IA)

Necrosis of any type is distinctly unusual Very occasionally a polymorphonuclear response may be observed (Plate IIC) and in such cases some tissue breakdown does occur Caseation necrosis is extremely rare Death of large masses of cryptococci in tissues may give rise to what superficially appears to be



FIG 39 — Human cryptococcal meningoencephalitis The subarachnoid space is markedly distended by the fungus and its mucinous capsular material Perivascular extension into the cerebral parenchyma is illustrated in *B* and *C* Compare with similar process in cat shown in Fig 30 *A* × 16, *B* × 100, *C* × 50 (AFIP Accession No 337884, contributed by Veterans Administration Hospital, San Francisco)

tissue caseation[15] (Fig. 43). Because of the usual absence of necrosis, secondary phenomena such as cavitation, abscess formation, and hemorrhage are infrequent

Two special groups of tissue reactions have been described in which fungus cells may or may not be present. One group includes sarcoid (Fig. 44) and sarcoid-like patterns (Fig. 41); the second Hodgkin's disease, other malignant

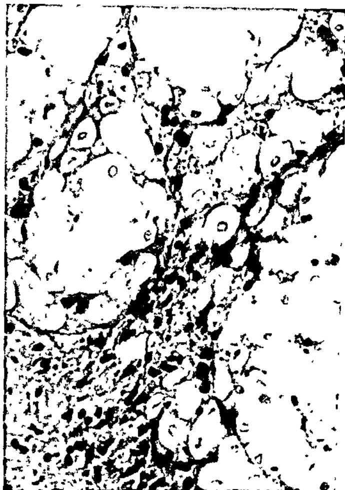


FIG 40—Thickly encapsulated cryptococci in a brain lesion Large numbers of macrophages are intermingled with the fungus cells. The distending growth of fungus cells within the cytoplasm of macrophages causes them to assume a signet ring appearance (arrows) or seemingly to disappear × 500 (AFIP Accession No 107139, contributed by Lawson General Hospital, Atlanta, Georgia)

lymphomas, and leukemia The relationship of cryptococcosis to the latter disorders is discussed on pages 35–36, wherein it is concluded that the cryptococcus is not the cause of any of the malignant diseases of the reticuloendothelial system. Furthermore, it is virtually inconceivable that any one infectious agent such as *C. neoformans* could provoke histologic reactions so diversified as to resemble those of Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, lymphocytic

Fig. 41.—Localized granulomatous pneumonitis due to *C neoformans* The lesion, first detected by routine chest x-ray, was surgically resected. Frozen section diagnosis of Boeck's ... d was confirmed ... en acid-fast or- ... not demon- ... e sarcoid-like ... ous Hema- ... sin. × 130. ... e of coarse ... some areas ... for fungus ... emonstrated ... ylin-eosin. ... h special ... many of ... found to ... icarmine- ... Muci- ... atoxylin ... (AFIP ... , con- ... l Hos- ...)

A

B

C

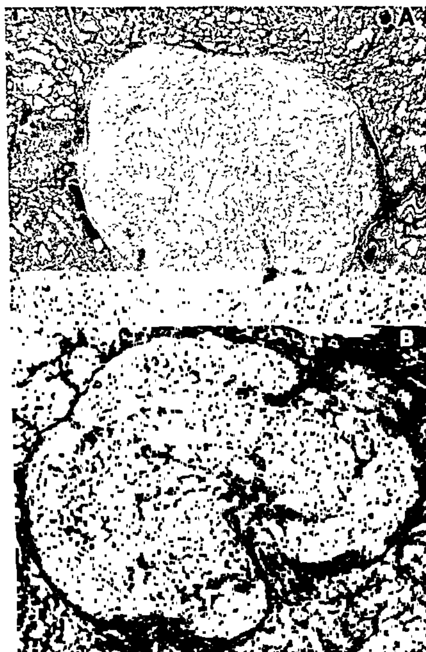
leukemia, monocytic leukemia, granulocytic leukemia, and multiple myeloma, all of which have been reported in association with cryptococcosis. On the other hand, it seems reasonable to assume that this fungus as well as other etiologic



Fig 42.—Large myxoid tumor-like mass in lung of a patient who died with extensive meningoencephalitis (See also Figs 11, 49, 50, 55.) The pulmonary alveoli are overdistended by the massive proliferation of fungus cells while host reaction is negligible. A × 5, B × 160. (AFIP Accession No 270523, contributed by Veterans Administration Hospital, Alexandria, Louisiana.)

factors may be responsible for the development of discrete, non-caseating, epithelioid tubercles typical of sarcoidosis.

Although cryptococcosis is generally a subacute or chronic infection, it produces relatively little fibrovascular proliferation in most tissues. It tends to be most pronounced in subpleural granulomas which probably represent healing of



[illegible] from one of the cases reported by Baker and Haugen[24] (*A* AFIP Accession No 706968, × 10, contributed by Dr Frederick C Zak, Pathologist, North Shore Hospital, Manhasset, New York *B* × 7, courtesy of Professor R. D. Baker.)

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Mycobacterium tuberculosis, Histoplasma capsulatum and *Coccidioides immitis* is infrequently encountered in cryptococcosis (Fig 47) and the productive fibrosis especially typical of actinomycosis is not observed. Significant calcification is most unusual, but spheroidal concretions in calcified lesions of other etiology may be misinterpreted as fungus cells (Fig. 82).

Two principal types of tissue lesions can be correlated with the duration of the disease, as reported in a pathologic study of 26 cases of cryptococcosis by Baker and Haugen.[15] Early lesions are gelatinous (mucinous) while older



FIG 45 –Subpleural granuloma interpreted as successful localization of primary cryptococcal granuloma Viable fungus cells were found in small foci within this lesion which was obtained by biopsy at thoracotomy Patient died of tuberculosis and autopsy revealed no other evidence of cryptococcosis × 30 (AFIP Accession No 510705, contributed by Veterans Administration Hospital, Memphis, Tennessee)

lesions are granulomatous Cryptococci are initially inert in tissues and form masses of organisms with but little surrounding inflammatory response, later they are engulfed by giant cells and macrophages Age, race and sex have no influence upon the character of tissue alteration, however, the presence of lymphoma lowers the resistance to infection.

The morphologic and histochemical characteristics of *Cryptococcus neoformans* in tissue sections are presented on pages 116–120 Included there are descriptions and illustrations of some of the structures which may be misinterpreted as cryptococci With the increasing interest in fungus diseases, a trend toward overdiagnosis has been noted and obvious artefacts illustrated as cryptococci have been published in case reports

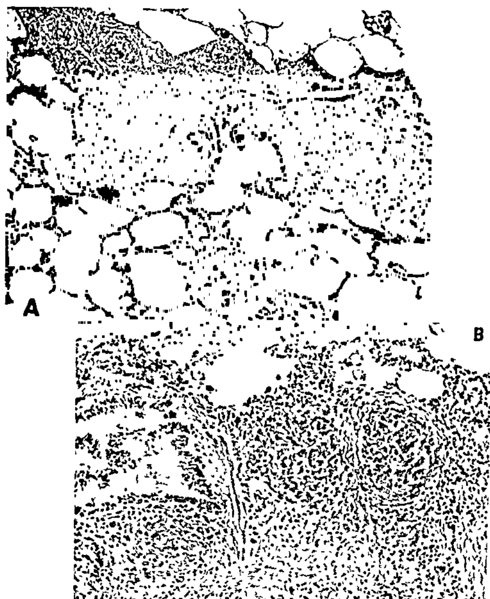


FIG 44 —Non-caseating sarcoidal granulomas of lung in a 41-year-old man who died of nervous system cryptococcosis The fungus was demonstrable in lesions of the brain and kidney but not in the sarcoid-like granulomas of lung and mediastinal lymph nodes This patient had an antecedent history of a respiratory infection with cough, fever, and malaise but without chest pain or hemoptysis for about two years before development of nervous system manifestations See chest x-ray (Fig 10) A × 33, B × 135 (AFIP Accession No 606695, contributed by Veterans Administration Hospital, Atlanta, Georgia)

the primary site of infection[172] (Fig 45) Granulation tissue (Fig 46) has been observed in localized cutaneous lesions but rarely pseudoepitheliomatous hyperplasia which is so characteristic of other mycoses, especially North American blastomycosis. Fibrous encapsulation of focalized lesions of the type caused by

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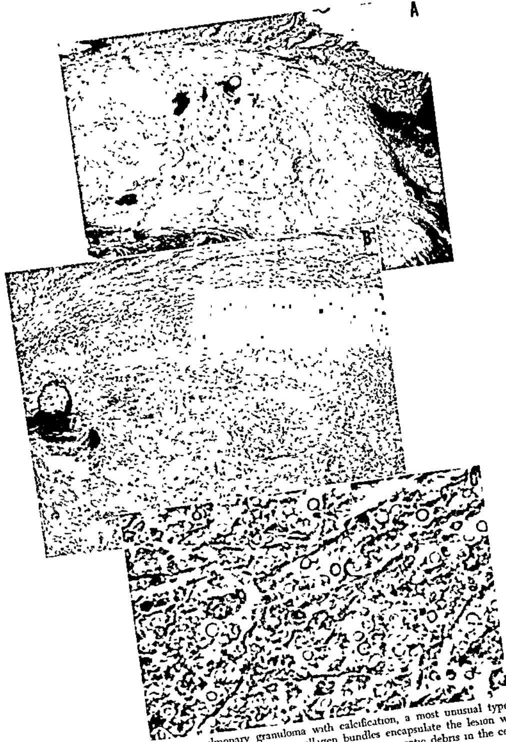


Fig 47 —Fibrocaseous pulmonary granuloma with calcification, a most unusual type of lesion produced by *C neoformans* A Dense collagen bundles encapsulate the lesion while a band of histiocytes forms the inner wall ×20 *B* There is necrotic debris in the center in which viable cryptococci are found ×50 C P.A S stain ×500. (AFIP Accession No 654575 contributed by Valley Forge Army Hospital)

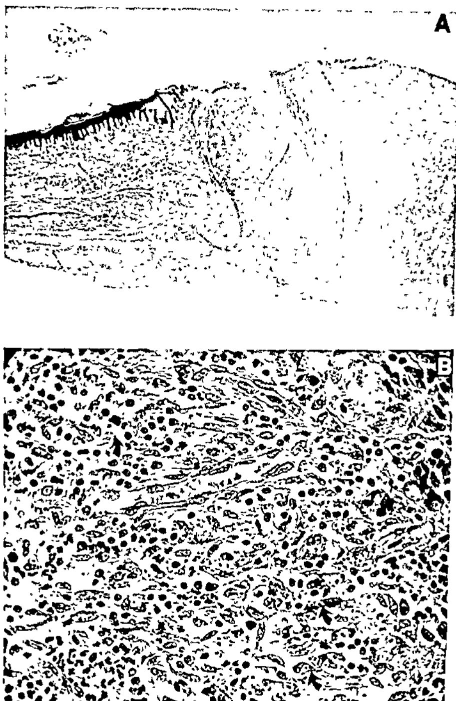
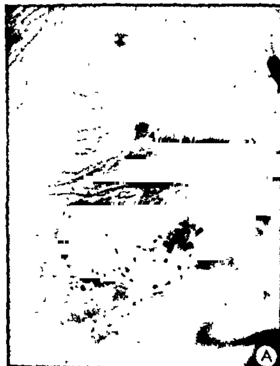


FIG 46 —Cutaneous ulceration secondary to osteomyelitis of underlying bone, same case illustrated in Fig 22 *A* There is a noteworthy lack of pseudoepitheliomatous hyperplasia of the epidermis at the edge of the ulcer *B* An exuberant proliferation of granulation tissue is observed in the subcutaneous tissue The fungus cells, present as minute intracellular parasites (arrows), could be mistaken for *Histoplasma capsulatum*, but typical staining with mucicarmine and demonstration of characteristic forms in other areas permitted an unequivocal etiologic diagnosis *A* × 13, *B* × 350 (AFIP Accession No 606561)


A


B


C


D


E


F


G


H

1. PULMONARY CRYPTOCOCCOSIS

Gross examination may reveal widespread pulmonary involvement or a localized process. *With dissemination, miliary tubercles resembling those of* tuberculosis are observed (Fig. 48) but when examined closely they appear more mucoid than those of tuberculosis. Solitary cryptococcal masses in the lung, usually ranging from 2 to 7 cm in diameter, are found at the lung periphery (Fig. 3), close to the hilum (Fig. 49), or in the middle of a lobe When the tissue reaction is great and the relative number of organisms small, the masses

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PLATE I. MACROSCOPIC LESIONS IN CRYPTOCOCCOSIS

A—Extensive cutaneous cryptococcosis in a 31-year-old woman with granulocytic leukemia Acneform lesions have ulcerated and coalesced with others to involve a broad area of the skin (See also Fig. 19)

B—Granulomatous chorioretinitis due to *C neoformans* The eye was enucleated because of severe ocular pain and loss of vision There were no other symptoms of cryptococcosis *in the 71-year-old woman* There is great thickening of the retina, which is primarily involved, while choroidal inflammation appears to be secondary (Case reported by DeBuen et al[48] AFIP Accession No 599915, contributed by Dr T P McKee, Johnson City, Tennessee) (See also Fig 66)

C—Extensive bilateral involvement of adrenals in a 33-year old man with brownish discoloration of the skin suggestive of adrenal insufficiency (Case reported by Rigdon and Kirksey[143] Courtesy of Dr R H Rigdon, Professor of Pathology, University of Texas, Galveston AFIP Accession No 697988)

D—Multiple small discrete subpleural granulomas in a young woman who died of cryptococcal meningoencephalitis (AFIP Accession No 261869 contributed by Dr W S Randall, formerly Pathologist, Ochsner Clinic, New Orleans, Louisiana)[101, 141]

E—Ventral surface of same brain shown in Fig 53A Glistening filmy bands of exudate in the leptomeninges envelope and distort several of the cranial nerves (AFIP Accession No 261869)[101, 141]

F—Miliary granulomas over ependymal surface of lateral ventricle Gross specimen magnified 6 ×. (AFIP Accession No. 548771, contributed by Tripler Army Hospital, Honolulu, Hawaii) The patient, a Japanese-American veteran of World War II became acutely ill, with chills, fever, and temporal headache When first admitted he showed no signs of meningitis Because of a spiking fever, malaria was suspected but smears were negative for malarial parasites Four days after admission, signs of meningitis were evident and there was spinal fluid pleocytosis Sugar was 55 mg per cent There were 661 cells with 90 per cent neutrophiles Bacterial meningitis was suspected and intensive antibiotic therapy started The clinical course was that of tuberculous or mycotic meningitis India ink preparations of a subsequent spinal fluid specimen revealed yeast-like cells Cultures of spinal fluid revealed *C neoformans* India ink preparations of sputum also showed the fungus Chest x-ray films disclosed marked pleural thickening *on the right and mediastinal shift to the* right The patient died five and one-half weeks after admission and less than two months after onset of first symptoms

G and *H*—Extensive cryptococcal meningoencephalitis *G*—Multiple minute cystoid lesions are observed along the natural surface of the cerebral convolutions (soapsuds appearance) as well as on the cut surface of the brain *H*—Much larger deep-seated lesions are shown in the basal ganglia As a result of the pronounced perivascular proliferation of organisms, the cerebral blood vessels appear as rigid tubes within cavernous channels Both specimens magnified 6 ×. (AFIP Accession No 270523, contributed by Veterans Administration Hospital, Alexandria, Louisiana)



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D—Multiple small discrete subpleural granulomas in a young woman who died of cryptococcal meningoencephalitis (AFIP Accession No 261869 contributed by Dr W S Randall, formerly Pathologist, Ochsner Clinic, New Orleans, Louisiana)[101, 141]

E—Ventral surface of same brain shown in *Fig. 53A* Glistening filmy bands of exudate in the leptomeninges envelope and distort several of the cranial nerves (AFIP Accession No 261869)[101, 141]

F—Miliary granulomas over ependymal surface of lateral ventricle Gross specimen magnified 6 × (AFIP Accession No 518771, contributed by Tripler Army Hospital, Honolulu, Hawaii) The patient, a Japanese-American veteran of World War II became acutely ill, with chills, fever, and temporal headache When first admitted he showed no signs of meningitis Because of a spiking fever, malaria was suspected but smears *were negative for malarial parasites* Four days after admission, signs of meningitis were evident and there was spinal fluid pleocytosis Sugar was 55 mg. per cent. There were 661 cells with 90 per cent neutrophiles. Bacterial meningitis was suspected and intensive antibiotic therapy started The clinical course was that of tuberculous or mycotic meningitis India ink preparations of a subsequent spinal fluid specimen revealed yeast-like cells Cultures of spinal fluid revealed *C neoformans.* India ink preparations of sputum also showed the fungus Chest x-ray films disclosed marked pleural thickening on the right and mediastinal shift to the right The patient died five and one-half weeks after admission and less than two months after onset of first symptoms

G and *H*—Extensive cryptococcal meningoencephalitis *G.*—Multiple minute cystoid lesions are observed along the natural surface of the cerebral convolutions (soapsuds appearance) as well as on the cut surface of the brain *H*—Much larger deep-seated lesions are shown in the basal ganglia As a *result of the pronounced perivascular proliferation* of organisms, the cerebral blood vessels appear as rigid tubes within cavernous channels Both specimens magnified 6 ×. (AFIP Accession No 270523, contributed by Veterans Administration Hospital, Alexandria, Louisiana)

are firm and rubbery and present a non-specific granulomatous appearance (Fig. 3) On the other hand, with massive accumulations of cryptococci and a relative lack of tissue response, the pulmonary mass may have a mucoid character (Fig 49). Solitary cryptococcal lesions in the lung come to the attention of the pathologist either as surgical specimens, operation having been performed for a mass in the lung, or at autopsy of a patient who died of cryptococcal meningitis

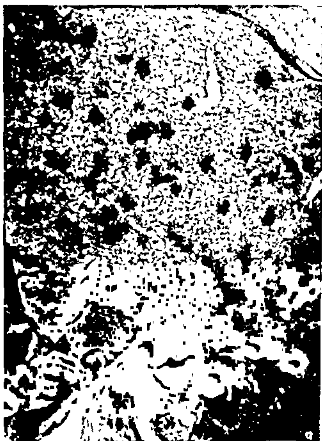


FIG. 48 —Miliary collections of cryptococci in the lungs of a 65-year-old Negro who had chronic lymphocytic leukemia The lesions in this case consisted almost entirely of fungus cells with negligible tissue response Case 25 of Baker and Haugen[11] (Courtesy of Prof R D Baker)

Similar solitary lesions also may be found incidentally at autopsy The most common pulmonary lesion at autopsy was reported by Haugen and Baker[112] to be a subpleural nodule less than 1 5 cm in diameter

Lesions appearing radiologically as solitary lung tumors (Fig 3) seldom are diagnosed before surgical removal, and the pathologist must make the diagnosis by means of frozen sections This method is effective with the large mucoid lesions which suggest myxomatous neoplasms (Figs 42 and 49) since they contain large numbers of cryptococci In the more densely granulomatous lesions, organisms may be so few that they escape detection both by frozen and paraffin section technics. It is in such instances that special fungus stains and cultures are most valuable.



FIG 50.—Pulmonary cryptococcosis A Fungus cells and macrophages fill the pulmonary alveoli but the septae remain intact × 220 (AFIP Accession No 268527) B Larger accumulations of cryptococci have formed as a result of septal disintegration and rupture × 160 (AFIP Accession No 270523)

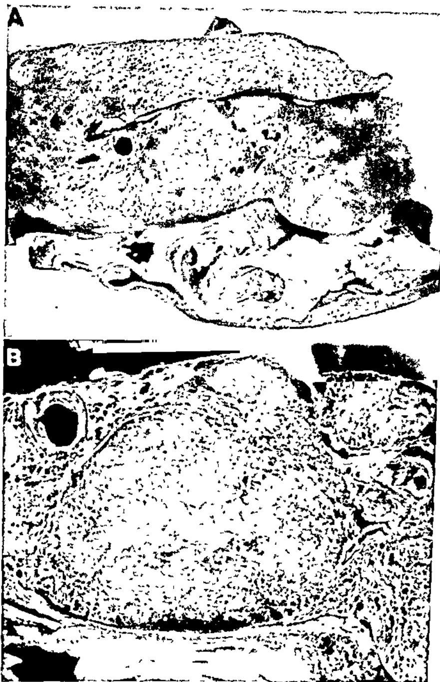


FIG 49—*A* This large discrete area of cryptococcal consolidation is situated centrally within the lung close to large blood vessels and bronchi *B* The myxomatous character of the cut surface of the lesion is apparent in a segment of *A* magnified 1½ × actual size See also Figs 11, 42, 50 and 55 (AFIP Accession No 270528)

only coarse vacuolization, special stains are required to demonstrate the presence of cryptococci within these vacuoles (Fig 41).

Even though the infection may be pneumonic, complications such as pleuritis with effusion (Fig 4) are uncommon. Organisms, however, have been found in the pleural exudate and in such cases the pleura may be studded with small pale nodular lesions. Only exceptionally is there direct extension of the disease process to the chest wall, mediastinum, or neck.[304] Bronchopleural and thoracic fistulas are extremely rare in this mycosis

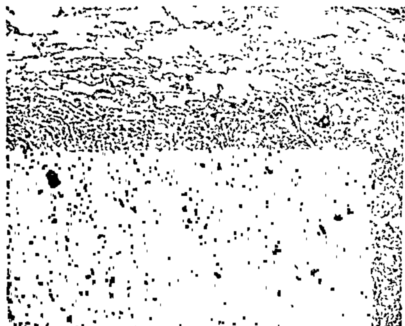


Fig 51 —Although cryptococcal granuloma of the lung is rarely encapsulated, the periphery of the lesion often shows a remarkably abrupt change from granulomatous inflammation to normal lung tissue The lesion in this case was obtained by surgical resection The patient, a 66-year-old man had a cold with cough, fever and weakness, chest x-rays revealed a 2 7 cm mass projecting from the right hilum Differential diagnosis included carcinoma, syphilitic gumma, tuberculosis, and fungus infection On microscopic examination Boeck's sarcoid was considered as a diagnosis until cryptococci were demonstrated × 50 (AFIP Accession No. 480736, contributed by Dr R H Fuller, Pathologist, St Mary's Hospital, Tuscon, Arizona)

Disseminated pulmonary cryptococcosis is almost invariably associated with widespread involvement of other tissues and rarely is encountered as a purely respiratory disease Miliary tubercles may be observed macroscopically, but more often their presence is not detected until the tissues are studied histologically. Microscopic examination reveals small clusters of organisms in almost every field Usually there is little or no reaction to these fungus cells and the impression obtained is that hematogenous dissemination was terminal In some cases, however, a histiocytic response is noted and a few well-developed granulomas containing giant cells may be seen among the miliary lesions

Pathologic changes in the lungs may be complicated by the coexistence of

Relatively small solitary granulomas of the lung in their early stages of development are peripheral and though subpleural in location, (Figs. 2 and 45) they rarely excite significant pleuritis (Fig 37). On microscopic examination of these lesions, the fungus cells are observed to fill and distend the pulmonary alveoli (Fig. 50). There is outpouring of histiocytic cells into the alveoli and many of the organisms are found within the cytoplasm of macrophages, others remain extracellular. At first little damage is done to the alveolar septa, but as the organisms proliferate and distend the alveoli, the pulmonary septa rupture (Figs 42 and 50*B*) As a result, larger collections of fungus cells are formed and on gross examination the cut surface of such a lesion may contain an abundant mucoid exudate Slight thickening of the septa is due to accumulation of mononuclear cells. Hyperemia is rarely pronounced and diapedesis of red cells into the alveoli usually is not seen

Larger pulmonary lesions, present longer than those just described, still may have a similar microscopic appearance. Pleural reaction may be negligible and the pulmonary alveolar walls often remain intact. About the periphery of the lesions, the transition from granuloma to normal lung is often abrupt, but in contradistinction to tuberculoma, is without fibrous encapsulation (Fig. 51). Macrophages tend to be larger and their cytoplasm frequently is filled with mucicarminophilic droplets, representing capsular mucopolysaccharide that has been engulfed by these phagocytes. Relatively little tissue necrosis is seen, even in the very large granulomas (Fig 52) This is in contrast to the focalized pulmonary lesions of coccidioidomycosis and histoplasmosis in which coagulative necrosis is characteristic, and the lesions of North American blastomycosis in which microabscess formation is the rule. The paucity of necrosis explains the infrequency of pulmon
countered, however, in
culosis or in which the
scattered microabscesses containing very few fungus cells mistakenly may arouse suspicion of North American blastomycosis Discrete lesions in the lungs showing a peculiar type of caseation have been described by Baker and Haugen[15] (Fig 43) In these there is a massive accumulation of dead fungus cells with but little tissue reaction This is in contrast with ordinary caseation necrosis in which the tissue elements rather than the causative agent become necrotic.

Fibrosis and an abundance of giant cells may be seen about the periphery of some cryptococcal granulomas, but only rarely, if ever, does the lesion resemble the classic "tuberculoma' with its concentric rings and laminated collagen fibers Even though the cryptococcal granuloma may be discrete and subpleural (Figs 2 and 45), it lacks the pleural callus and other distinguishing features of the pulmonary tuberculoma described by Mahon and Forsee[278] and others.[35,473]

Scattered epithelioid tubercles are sometimes seen and in an occasional case they may be so numerous and prominent that a diagnosis of sarcoidosis is suggested (Fig 41) As a rule those granulomas with more fibrosis, giant cells and epithelioid tubercles contain fewer organisms Almost all of the fungus cells are intracellular, particularly within giant cells (Figs. 38 and 41). Since in hematoxylin and eosin-stained sections the cytoplasm of giant cells may show

disease and cryptococcosis coexist in the same lung, the problem of diagnosis is more difficult. The fungus may actually infect areas involved by the lymphoma and in such cases the entire pathologic process erroneously may be attributed to cryptococcosis. On the other hand, when portions of the lung are affected separately by the lymphoma and the fungus, relatively little difficulty is experienced in differentiating the resulting lesions.

2. CRYPTOCOCCAL MENINGITIS

In many fatal cases of cryptococcosis macroscopic alterations are found only in the nervous system and even here they may be minimal. The brain and

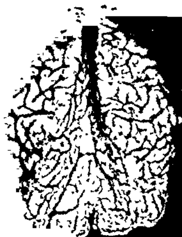
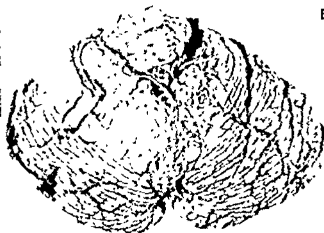


FIG. 53.—A. Cryptococcal meningitis. There is intense congestion of the subarachnoid vessels but minimal exudate in the leptomeninges over the cerebral hemispheres. The cerebellar convolutions are obscured by thick membranous exudate in the subarachnoid space. *The densely granulomatous character of this meningeal reaction is illustrated in Fig. 57.* (AFIP Accession No. 281869, contributed by Dr. W. S. Randall, formerly Pathologist, Ochsner Clinic, New Orleans.) *B.* Part of the dorsal surface of the cerebellum is covered by a plastic meningeal exudate. See also Plate 1E. (AFIP Accession No. 548771.)



meninges are hyperemic and there is slight flattening of the cerebral convolutions. Meningeal reaction is often most pronounced over the base of the brain and dorsal surface of the cerebellum where the membranes lose their trans-

other diseases. Patients with cryptococcal meningoencephalitis frequently have non-mycotic pneumonitis caused by bacteria or aspiration of foreign material These lesions differ from those of cryptococcosis in their purulent and necrotizing characteristics Tuberculosis and cryptococcosis have been found to coexist in



FIG 52—A In most pulmonary lesions of cryptococcosis the reticulum framework of the lung shows relatively little alteration Reticulum stain, × 60 (AFIP Accession No 557542) B In some cases tissue necrosis and cavitation may be observed, hematoxylin-eosin, × 50 (AFIP Accession No 337884)

the same lungs. True caseation necrosis, characteristic of tuberculosis, is not observed in cryptococcosis

Lesions of cryptococcosis are known to co-exist with those of coccidioidomycosis, histoplasmosis and nocardiosis In every instance the histologic diagnosis has been possible because of the characteristic tissue reactions and the demonstration of causative organisms in the lesions. However, when Hodgkin's

associated with a granulomatous cellular response, the membranes may adhere to the cortex. In some cases of prolonged duration or following intensive intrathecal therapy with antimycotic agents, fibrosis of the meninges is prominent (Figs 53 and 56).



FIG 55 —A Extensive meningoencephalitis, same case illustrated in Figs 11, 42, 49 and 50. There is variable thickening of the meninges and distention of the subarachnoid space by the characteristic mucinous exudate. Multiple minute cystoid spaces filled with cryptococci and their capsular polysaccharide are distributed along the cerebral gray matter. B Enlarged 4 ×. (AFIP Accession No 270523.)

Microscopically, the degree of the meningeal reaction varies from one area to another. Frequently there are foci in which the subarachnoid space is distended by multitudinous organisms and the cellular reaction is minimal. An

parency, become thickened and obscure the underlying brain parenchyma (Fig. 53).

The subarachnoid space is characteristically distended by a light grayish, adherent exudate which may have a distinctly mucoid appearance. Sometimes the brain surface appears as though covered by tiny soap bubbles (Plate I*G* and *H*). Even though the leptomeninges over the cerebral hemispheres show minimal change, there may be significant alterations about the brain stem and basal cisterns. The exudate may cover the base of the brain and superior surface of the cerebellular hemispheres in a uniform manner (Fig. 53) or there may be



FIG 54.—Chronic cryptococcal leptomeningitis in a 55-year-old white man who had frequent convulsive seizures for more than four years Ventriculography showed internal hydrocephalus and evidence of a tumor in the posterior fossa A glistening plastic exudate adheres to the optic chiasm, basilar artery, and brain stem (AFIP Accession No 265063, contributed by Dr W B Dublin, formerly Pathologist, Indianapolis General Hospital, Indianapolis Indiana) (See also Fig 56)

patchy involvement of such structures as the chiasm (Fig. 54), the entrance to the sylvian fissure, the gasserian ganglion, or certain cranial nerves (Fig 55 and Plate I*E*)

Sometimes tumor-like formations develop in the meninges or minute tubercles occur along the course of small blood vessels In rare instances meningeal involvement can be traced to direct extension through the skull from lesions in the middle ear, accessory nasal sinuses or orbit

When the subarachnoid space is filled with a mucoid exudate, the membranes can be lifted off with ease When this is done, the surface of the cerebral convolutions may reveal fine dimpling, each dimple representing the site of a cystoid lesion formed by a collection of fungus cells and their mucoid capsular substance. If the meningeal reaction is of the patchy type, and especially if it is



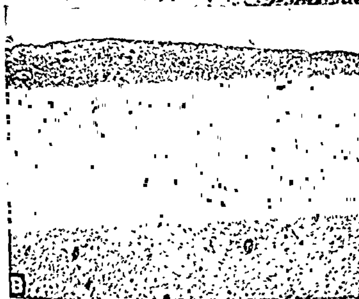


FIG. 57.—Granulomatous leptomeningitis due to *C. neoformans*. The subarachnoid space is filled with a dense accumulation of chronic inflammatory cells including a large number of foreign body type giant cells. The fungus recovered from this patient's spinal fluid was extremely sensitive to low concentrations of Actidione. This was the first case treated with Actidione.[illegible] During those periods when the patient was receiving the drug, cultures of spinal fluid showed a lowered colony count, there was less fever, the spinal fluid pressure was lower, and papilledema regressed. When 8 consecutive spinal fluid cultures were negative, it seemed that the infection was controlled. The patient, however, had Jacksonian seizures, became stuporous, then comatose, and died. See also Fig. 53A and Plate IE. (AFIP Accession No. 261869, contributed by Dr. W. S. Randall, formerly Pathologist, Ochsner Clinic, New Orleans.)

adjacent microscopic field often shows a prominent histiocytic response, most of the organisms being found within the mononuclear inflammatory cells Giant cells are invariably present, though their numbers may be great or small

In some cases the meningeal reaction is densely granulomatous with an abundance of giant cells (Fig. 57). The fungus cells are always less numerous in reactions of this type, but usually they can be demonstrated without a prolonged search. Special stains for fungi are especially valuable in such cases. Occasionally, when the cryptococcal forms are extremely few their differentiation from *B. dermatitidis* or *C. immitis* is difficult, but special stains for mucin will assist in the differential diagnosis (See Appendix, formula 18, and Plate IVC and *E*).



Fig 56 – Pronounced fibrovascular proliferation in the meninges of a man who had convulsive attacks for four years See also Fig 54 (AFIP Accession No 265063)

While the cryptococcus elicits a typical macrophagic cellular response, focal exudation of polymorphonuclear leukocytes is observed in rare cases (Plate IIC) This reaction, however, is seldom of a degree that would lead to confusion with the acute bacterial meningitides Moreover, the characteristic cryptococcal forms are found among the pus cells, so that an etiologic diagnosis can be made

3 CRYPTOCOCCAL ENCEPHALITIS

Diffuse involvement of the neural parenchyma may develop in either of two ways The first is the result of extension of the meningeal infection along the perivascular sheaths into the brain substance (Figs 39, 55 and Plate IG and H) Proliferation of organisms therefore may occur at varying depths be-

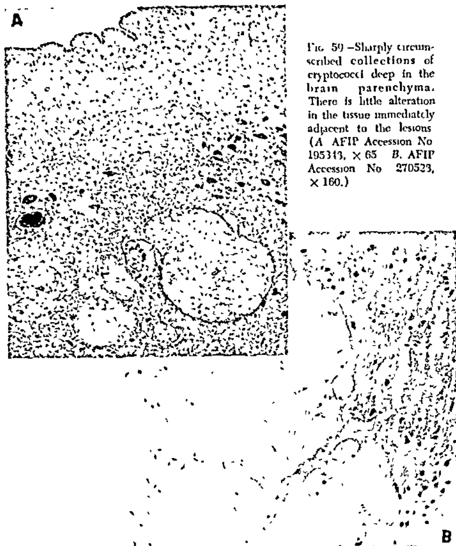


Fig. 59 —Sharply circumscribed collections of cryptococci deep in the brain parenchyma. There is little alteration in the tissue immediately adjacent to the lesions (*A* AFIP Accession No 195313, × 65 *B*, AFIP Accession No 270523, × 160.)

neath the brain surface so that the sectioned brain appears irregularly studded with cysts of pin-point to pin-head size, found predominantly in the gray matter On microscopic examination, some sections will show the cystoid lesion communicating with the subarachnoid space by a long narrow perivascular zone of involvement, the entire lesion presenting a flask-shaped contour (Fig. 39)

The second mechanism by which parenchymatous lesions are produced is embolism [135] Embolic lesions are best recognized when deep-seated When superficial, they are not distinguishable from those produced by direct extension from the meninges Collections of cryptococci found beneath the ependyma, in the periventricular and periaqueductal gray matter, in the basal ganglia, in the dentate nucleus of the cerebellum, and in the white matter of the cerebral hemispheres are almost certainly embolic (Fig 58)



FIG 58 —Deep-seated lesions believed to represent hematogenous localization of cryptococci A Minute cystoid foci are seen in the cerebral cortex but the basal ganglia are much more extensively involved In addition, a larger discrete mass with glistening mucoid appearance is present in the lentiform nucleus and internal capsule on one side B The cerebellar cortex is spared but typical lesions are observed in the dentate nucleus. (AFIP Accession No 195343, contributed by Brooke Army Hospital)

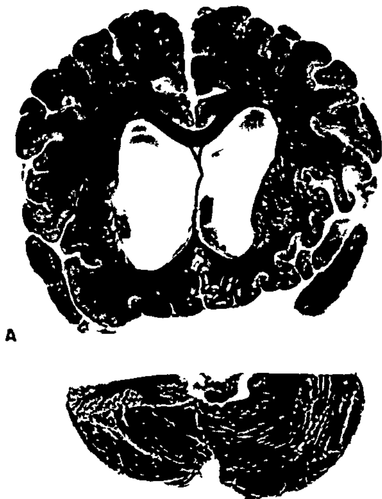


FIG 61 —Extreme ventricular dilatation and cortical atrophy due to severe long-standing cryptococcal meningoencephalitis Ventriculograms were interpreted as showing evidence of a tumor in the posterior fossa. See also Figs 54, 56 and 62 (AFIP Accession No 265063, contributed by Dr W B Dublin, formerly Pathologist, Indianapolis General Hospital, Indianapolis, Indiana)

gal masses, which accounts for the cystic appearance of the lesions in fixed specimens The cyst formation had once been attributed to a histolytic action of the fungus, hence the name *Torula histolytica,* but such activity has never been proved. Freeman and Weidman grew the fungus for weeks on sterile brain without observing digestion of tissue [135,137] Furthermore, compression of the adjacent tissues without the excitation of a significant inflammatory re-



FIG 60—Deep-seated parenchymatous lesions of extremely granulomatous character See also Fig 57 (AFIP Accession No 261869)

Parenchymatous lesions, whether produced by embolism or by extension of the microorganism from the meninges, are typically small, cystoid and perivascular (Fig. 39). When fresh brain tissue is sectioned these lesions are found to be filled with mucoid material representing the accumulated capsular polysaccharide of the fungus cells. Formalin fixation causes shrinkage of the fun-



FIG 63—Obliterative endarteritis secondary to granulomatous cryptococcal meningitis A A discrete granuloma in the meninges of the spinal cord surrounds an artery (arrow) that shows pronounced narrowing of its lumen as a result of intimal proliferation × 60 (AFIP Accession No 159793, contributed by Letterman General Hospital, case reported by Gifford and Hullinghorst[184] B An extremely granulomatous reaction in the leptomeninges of a patient who was thought to have tuberculous meningitis Arteries shown in the upper right and lower left corners of the illustration have markedly thickened intimal coats × 125 (AFIP Accession No 232094, contributed by Veterans Administration Hospital, Vancouver, Washington)

sponse is more in keeping with the concept of an expanding spherical mass of fungus cells. Tissue elements, including nerve cells and fibers, are seen in the cyst wall in intimate relation with the encapsulated organisms (Fig. 59). The nuclei and cytoplasmic granules of the adjacent nerve and ganglion cells are preserved.[136] Inflammatory cells other than macrophages are very few (Fig. 59). Fungus cells and an abundance of capsular mucopolysaccharide form the contents of the cysts.

Parenchymatous lesions of two other types are seen in addition to nonreactive cystoid lesions. One is a dense granulomatous process similar to that found in the meninges (Fig. 60). *Fungus cells are fewer and there is an abundance* of mononuclear elements and inflammatory giant cells. Varying degrees



FIG. 62.—Granulomatous thickening of choroid plexus (arrows) in widely dilated lateral ventricle. Same brain shown in Fig. 61. (AFIP Accession No. 265063.)

of gliosis are seen in such lesions. The second type resembles encephalomalacia (Plate II*D*). Under low power microscopy the neural parenchyma appears disrupted and edematous. *The area is heavily infiltrated by "gitter" cells. It* is in lesions of this type that the fungus cells are most easily overlooked, not because of their small numbers, for they are numerous, but because they are diffusely intermingled with microglia, a large proportion being located as single organisms within the cytoplasm of these cells.

Infections allegedly caused by *Cryptococcus neoformans* have been described in the newborn.[174, 294, 299, 401] These infants presented clinical, radiologic, and pathologic features characteristic of toxoplasmic encephalitis and chorioretinitis. Mineral concretions with striking similarity to cryptococci were, in our opinion, responsible for the erroneous diagnoses (Fig. 82). It is doubtful that these were other than *Toxoplasma gondii* infections.

A variety of secondary changes may be observed. Obstruction may occur anywhere in the ventricular system, as a result of which internal hydrocephalus develops (Figs. 14 and 61). Miliary granulations (Plate I*F*) or large masses producing filling defects in the ventricular system may be observed to project



FIG 63—Obliterative endarteritis secondary to granulomatous cryptococcal meningitis A A discrete granuloma in the meninges of the spinal cord surrounds an artery (arrow) that shows pronounced narrowing of its lumen as a result of intimal proliferation × 60 (AFIP Accession No 159793, contributed by Letterman General Hospital, case reported by Gifford and Hullinghorst[14] B An extremely granulomatous reaction in the leptomeninges of a patient who was thought to have tuberculous meningitis Arteries shown in the upper right and lower left corners of the illustration have markedly thickened intimal coats × 125 (AFIP Accession No 232094, contributed by Veterans Administration Hospital, Vancouver, Washington.)

into the ventricles from the subependymal gray matter or from the choroid plexus (Fig. 62).

When meningeal lesions predominate, there may be an external hydrocephalus and compression atrophy of the cerebral convolutions. In cases of long duration in which there have been repeated exacerbations and remissions, fibrosis and calcification will be found.[21] The meningeal vessels may show pronounced obliterative endarteritis (Fig. 63). Contraction of scar tissue may lead to added deformity of the brain and its ventricular system. In Case 3 of Mosberg and Arnold[291] in which the spinal fluid returned to normal after extensive and varied therapy, autopsy revealed no active infection but extensive secondary brain damage.

4. SPACE-OCCUPYING GRANULOMAS OF THE CENTRAL NERVOUS SYSTEM

Frequently one or more discrete tumor-like granulomas may be found in any part of the brain, cord, or their meninges (Figs 16 and 64). Lesions of this type account for the neurosurgical procedures performed on almost one-fourth of the reported patients with nervous system cryptococcosis[60] (see page 20). The granulomas are roughly spherical masses which may be deep-seated in the cerebral parenchyma, or located more superficially in the leptomeninges They may displace the ventricular system (Fig. 15) Space-taking dural granulomas even occur (Fig 64) and these may be associated with secondary changes in the cranial bones Only rarely does a meningeal lesion develop secondary to bone infection due to a primary process in the sinuses, middle ear or orbit.[223]

5 INVOLVEMENT OF SKIN AND MUCOUS MEMBRANES

Cryptococcal infection of these tissues in man is usually a manifestation of systemic cryptococcosis (Figs. 17–21) The fungus may localize and proliferate in the corium, or the skin may be involved by direct extension from an adjacent bone lesion (Figs 22 and 46). Draining sinus tracts from infected internal organs are seldom encountered in cryptococcosis In some cases there is a tremendous accumulation of fungus cells and their mucinous capsular material in and beneath the epidermis The epidermis becomes stretched and attenuated by the growth of organisms (Fig 65) Ordinarily in cryptococcosis there is little or no reactive hyperplasia of the epidermis (Figs 46 and 65) and the cancer-like proliferation, which is so characteristic of cutaneous blastomycosis is rare in this disease On the other hand, cryptococcosis of the soft tissues may mimic subcutaneous myxoma or lipoma both clinically and grossly (Fig. 18) The cut surface presents a glistening mucoid appearance but the tissue is of moderately firm consistency These cases are recognized microscopically as cryptococcal infections with little difficulty since the fungus cells are present in vast numbers

In other cases the cutaneous and subcutaneous tissues respond with a great



FIG 64—A granulomatous mass measuring 5 cm in diameter is attached to the dura that covers the floor of the middle fossa Multiple papillary excrescences of translucent tissue project from the surface of the mass. When the dura was stripped away from the bony floor of the middle fossa, openings into the inner table of the skull were disclosed The patient had had severe "sinus trouble" for a month, then developed fever, chills, and convulsive seizures Spinal fluid examination revealed the presence of "yeast" The patient became comatose and died Although autopsy revealed diffuse cryptococcal meningoencephalitis, this dural mass was the only lesion of tumor-like proportions (AFIP Accession No 63555, contributed by Lt Col G R. Callender, Walter Reed General Hospital)

amount of capsular polysaccharide, and when intracytoplasmic, may be confused with *Histoplasma capsulatum* (Fig 46) Mycologic and histochemical studies are required before an unequivocal diagnosis can be made

In both types of cutaneous response the overlying epidermis tends to become ulcerated The non-reactive lesions exude a mucinous material from which a diagnosis may be established readily by direct examination, culture or animal inoculation (see Laboratory studies, pages 96, 99 and 111)

6 CRYPTOCOCCAL INVOLVEMENT OF OTHER ORGANS

Although virtually any tissue may become infected by this fungus, localization rarely occurs elsewhere than in the lungs or nervous system, however, a few such cases are on record These localized lesions invariably cause considerable difficulty in both clinical and pathologic diagnosis Lesions in these unusual sites, as in the lung, are often densely granulomatous and contain relatively few organisms which may be overlooked or else mistaken for *Histoplasma capsulatum*



Fig 65 —Photomicrographs of one of the strawberry-like cutaneous eruptions illustrated in Fig 17 There is extensive infiltration and proliferation of cryptococci within and beneath the epidermis *A* There is ulceration of the epidermis and fungus cells may be seen on the surface of the skin as well as in and beneath the epithelium Periodic acid-Schiff reaction, ×80 *B* The massive growth of fungus cells has caused an extreme degree of attenuation and elongation of the epithelium but this is not active pseudoepitheliomatous hyperplasia of the type typical of North American blastomycosis Hematoxylin-eosin, ×60 *C* Proliferation of cryptococci within the epidermis Periodic acid-Schiff reaction, ×175 (AFIP Accession No 701626, contributed by Dr F Linell, Department of Pathology, Lund, Sweden[244, 249])

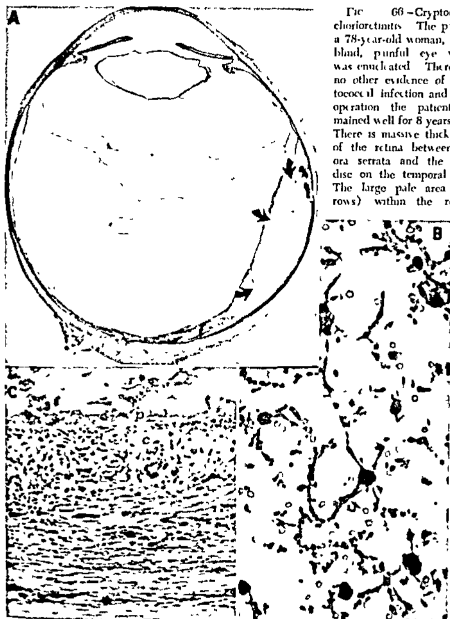


FIG 66 — Cryptococcal chorioretinitis The patient, a 78-year-old woman, had a blind, painful eye which was enucleated There was no other evidence of cryptococcal infection and after operation the patient remained well for 8 years *A* There is massive thickening of the retina between the ora serrata and the optic disc on the temporal side. The large pale area (arrows) within the retinal lesion represents a massive proliferation of fungus cells shown at greater magnification in *B* Hematoxylin-eosin, × 4 *B* The size of the lesion is due to a tremendous accumulation of cryptococci and abundance of capsular polysaccharide, with a paucity of inflammatory cells Hematoxylin-eosin, × 355 *C* The choroid (c) responds with a granulomatous reaction while the retina (r) and pigment epithelium (p) are necrotic × 225 Because of the mycelium-like strands of tissue debris shown in *B*, the fungus had been interpreted as *Candida* for several years Restudy of the sections with application of the newer staining technics, especially the mucicarmine stain, left no doubt that this fungus was in the genus *Cryptococcus* (AFIP Accession No 161194, contributed by Dr R J Gray, Pittsburgh, Pa) Case reported by DeBuen et al.[48] Compare with very similar case illustrated in Plate IB



FIG 65.—Photomicrographs of one of the strawberry-like cutaneous eruptions illustrated in Fig 17 There is extensive infiltration and proliferation of cryptococci within and beneath the epidermis *A There is ulceration of the epidermis and fungus cells may be seen* on the surface of the skin as well as in and beneath the epithelium Periodic acid-Schiff reaction, ×80 *B* The massive growth of fungus cells has caused an extreme degree of attenuation and elongation of the epithelium but this is not active pseudoepitheliomatous hyperplasia of the type typical of North American blastomycosis Hematoxylin-eosin, × 60 *C*. Proliferation of cryptococci within the epidermis Periodic acid-Schiff reaction, × 175 (AFIP Accession No 701626, contributed by Dr F Linell, Department of Pathology, Lund, Sweden.[illegible])

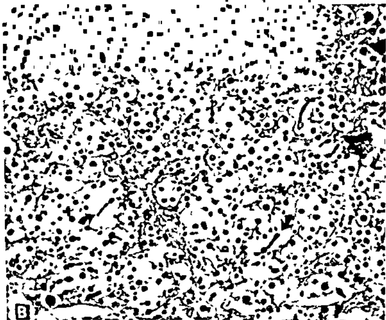


FIG 68—Splenic lesions from same case shown in Fig 67 *A* The large pale areas represent massive accumulations of cryptococci with minimal cellular response Hematoxylin-eosin, × 65 *B* Active proliferation is evident by the large numbers of budding fungus cells and the formation of elongated germ tubes (pseudomycelium) (arrow) Periodic acid-Schiff reaction, × 330

or another fungus We have been impressed with the difficulty in the histopathologic diagnosis of solitary cryptococcal granulomas of bone (Fig 46) and the eye (Fig 66)

Sometimes, in the course of dissemination of the cryptococci, one organ becomes more extensively involved than others The adrenals, for example, may be so massively destroyed that symptoms of Addison's disease are produced



Fig. 67.—Disseminated cryptococcosis in a patient with Hodgkin's disease. Minute lesions containing fungi, but few inflammatory cells, are present in many tissues (arrows) See also Fig 68 and Plate IIE. All sections stained with mucicarmine and counterstained with iron hematoxylin. (AFIP Accession No 152949, contributed by Walter Reed Army Hospital.)

A Liver × 100
B Lung × 250
C. Kidney × 250
D. Adrenal × 65

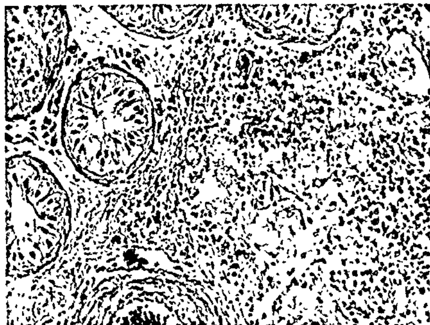


FIG 70 —Testicular lesion containing large numbers of small cryptococci. × 200 From same case as Plate IIF (AFIP Accession No 193343)

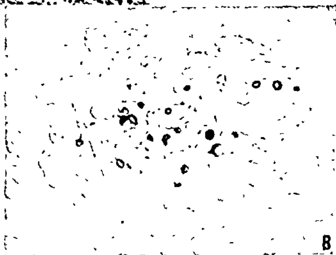
in the sinusoids of liver, spleen, bone marrow, and adrenal glands (Figs. 67 and 68, and Plate IIE and F) In fact, minute inflammatory foci, initially considered non-specific, which may be observed in practically all tissues examined (Figs. 27, 69 and 70) are often proved to contain cryptococci by special staining (Fig 69).

A large vegetation on the mitral valve and minute vegetations on the aortic valve, both produced by *C neoformans*, have recently been encountered at autopsy in a patient with chronic rheumatic heart disease (see page 151)

(Plate IC). In one case we have reviewed, the adrenal lesion at autopsy was thought to be a primary neoplasm and the gross appearance of the brain suggested metastatic tumor until microscopic examination revealed all lesions to be cryptococcal granulomas Similarly, massive lesions of bones may be misinterpreted as neoplasms or non-specific inflammation The gross appearance of these solitary cryptococcal lesions is seldom distinctive although the unusually



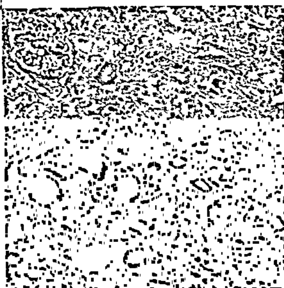
Fig. 69 —Cryptococcal myocarditis in a man with reticulum cell sarcoma *A* With hematoxylin and eosin stain the inflammatory cells obscure the few fungi present in the sections × 100

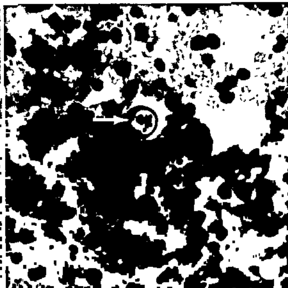


B With the Gridley stain, the inflammatory cells remain unstained and the cryptococci are readily found, × 400 (AFIP Accession No 232406, contributed by Army and Navy General Hospital, Hot Springs, Ark) (See also Figs 27 and 37)

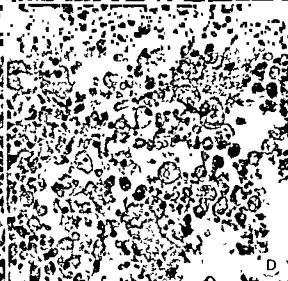
mucoid character of some masses (Fig 24C) should arouse suspicion of cryptococcosis

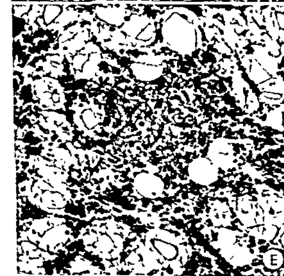
Enlargement of internal organs to the degree that they are clinically evident or obvious at autopsy, is decidedly unusual On the other hand, widespread microscopic visceral lesions seen only on histologic study are more frequently detected now than in the past Dissemination of an extreme degree is seen in those cases of malignant lymphoma or leukemia in which the fungus infection seems to be a terminal event (Figs 27 and 67–70, and Plate IIE and F) Small clusters of cryptococci are seen in the renal glomeruli (Plate IIA and Fig. 67),

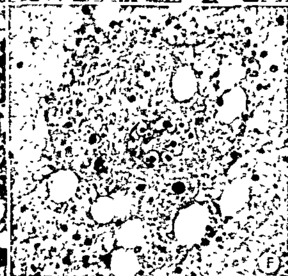





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A.—Mucinous casts stained pink by mucicarmine are observed in the renal tubules Note that pink-stained cryptococci are present in the glomeruli but not in the casts × 100 (AFIP Accession No 152949)

B.—Lipoidal granules stained brilliant red are present within the fungus cell (arrow). Frozen section of formalin-fixed mouse lung experimentally infected with *C neoformans* Oil red O stain, × 800 (AFIP Neg No 55–10380)

C.—An unusual necrotizing inflammatory response with intense exudation of polymorphonuclear leukocytes is seen about the pink-stained cryptococci Mucicarmine with hematoxylin, × 350. (AFIP Accession No 265063)

D.—Cryptococcal encephalomalacia Macrophages (stained yellow-brown) have phagocytized most of the pink-stained fungus cells Mucicarmine with hematoxylin, × 285 (AFIP Accession No 546861, contributed by Veterans Administration Hospital, Beckley, West Virginia)

E.—Small but widely disseminated collections of cryptococci are frequently observed in the bone marrow of those patients who have had a pre-existing malignant lymphoma or leukemia. The fungus cells are stained pink by mucicarmine Repeated nitrogen mustard and roentgen therapy account for the severe depletion of normal marrow elements. × 185 Same case illustrated in Figs 67 and 68 (AFIP Accession No 152949)

F.—This section of a bone-marrow fragment (stained by periodic acid-Schiff reaction) was obtained by sternal marrow aspiration from a patient who had received x-ray, nitrogen mustard, methyl bis-amino-hydrochloride, and triethylene melamine for Hodgkin's disease The cryptococci, stained here an intense red, were found quite unexpectedly in the marrow smears and sections. The fungus was also demonstrated subsequently in the spinal fluid, urine, and blood (AFIP Accession No 195343 Marrow smears and sections contributed by Col H A Van Auken and Maj J. H Akeroyd, Brooke Army Hospital) (See also Figs 58, 59 and Plate IIA)

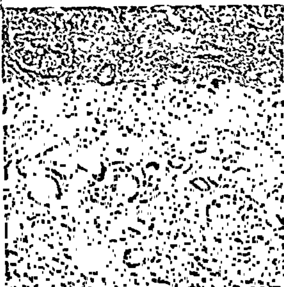
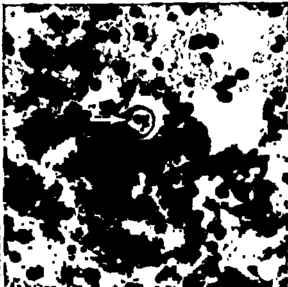
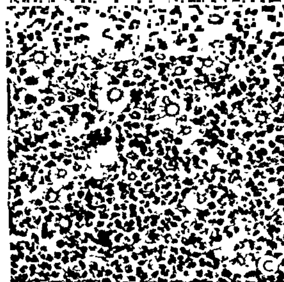
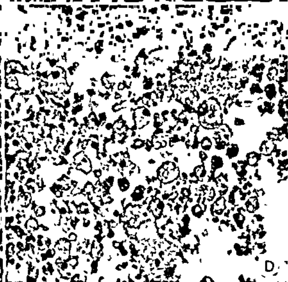
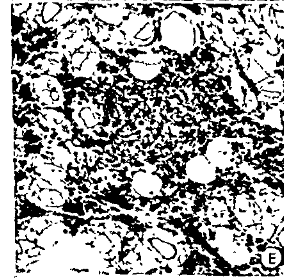
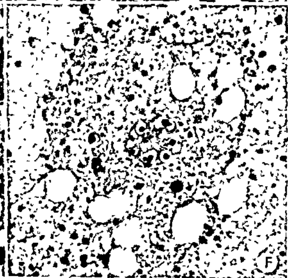



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PLATE II. *C NEOFORMANS* IN TISSUE SECTIONS

A—Mucinous casts stained pink by mucicarmine are observed in the renal tubules Note that pink-stained cryptococci are present in the glomeruli but not in the casts × 100 (AFIP Accession No 152949)

B—Lipoidal granules stained brilliant red are present within the fungus cell (arrow) Frozen section of formalin-fixed mouse lung experimentally infected with *C neoformans* Oil red O stain, × 800 (AFIP Neg No 55–10580)

C—An unusual necrotizing inflammatory response with intense exudation of polymorphonuclear leukocytes is seen about the pink-stained cryptococci Mucicarmine with hematoxylin, × 350 (AFIP Accession No 265063)

D—Cryptococcal encephalomalacia Macrophages (stained yellow-brown) have phagocytized most of the pink-stained fungus cells Mucicarmine with hematoxylin, × 285 (AFIP Accession No 546861, contributed by Veterans Administration Hospital, Beckley, West Virginia)

E—Small but widely disseminated collections of cryptococci are frequently observed in the bone marrow of those patients who have had a pre-existing malignant lymphoma or leukemia The fungus cells are stained pink by mucicarmine Repeated nitrogen mustard and roentgen therapy account for the severe depletion of normal marrow elements × 185 Same case illustrated in Figs 67 and 68 (AFIP Accession No 152949)

F—This section of a bone-marrow fragment (stained by periodic acid-Schiff reaction) was obtained by sternal marrow aspiration from a patient who had received x-ray, nitrogen mustard, methyl bis-amino-hydrochloride, and triethylene melamine for Hodgkin's disease The cryptococci, stained here an intense red, were found quite unexpectedly in the marrow smears and sections The fungus was also demonstrated subsequently in the spinal fluid, urine, and blood (AFIP Accession No 195343 Marrow smears and sections contributed by Col H A Van Auken and Maj J H Akeroyd, Brooke Army Hospital) (See also Figs 58, 59 and Plate IIA)

IX. Laboratory Studies

General laboratory studies, nonspecific in nature, are seldom helpful in the diagnosis of cryptococcosis. Alterations in the formed elements of the blood or in its chemical composition rarely become pronounced. There may be a mild degree of anemia and leukocytosis Significant deviations when present usually indicate a primary hematologic disorder of some other etiology. Failure to note this fact has led to the erroneous conclusion that the cryptococcus can provoke a leukemoid reaction or cause profound anemia. Likewise, eosinophilia, when present in a case of cryptococcosis, should be attributed to some other process. As with most infections, the blood sedimentation rate is increased

An increased utilization of specific laboratory procedures could result in discovery of many cases that often remain unrecognized and undiagnosed until pathologic studies reveal the mycotic nature of the disease. Examination of the sputum is particularly important The diagnosis of localized cryptococcal granuloma of the lung has rarely been made prior to thoracotomy, yet pathologic examination of resected tissues indicates that diagnoses would be possible more often and earlier if appropriate studies had been undertaken. It is not difficult to demonstrate fungus cells within the exudate contained in the lumens of bronchi (Fig 71) It is our impression that all too often sputum is examined only for acid-fast bacilli and tumor cells, and that other possible causes of localized lung lesions are disregarded during the preoperative "work-up" Perhaps this is due to a misconception that laboratory studies required to establish the diagnosis of mycotic disease are too specialized and too expensive for the average clinical laboratory This certainly is not the case, especially in cryptococcosis, since fungus cells can be demonstrated by direct examination of tissue fluids and exudates even more readily than tubercle bacilli or neoplastic cells. Furthermore, culture studies are no more difficult and are even less time-consuming than for *M tuberculosis* The notion held by many physicians and technicians that all yeasts recovered from the sputum are nonpathogenic accounts for other missed diagnoses.

The most important laboratory procedure in suspected cases of cryptococcal meningitis is examination of the spinal fluid The fluid is usually obtained under increased pressure and, in the early stages of the disease, it is clear and may contain only a small number of white blood cells and cryptococci Upon subsequent withdrawals and with progressive disease, the spinal fluid becomes turbid or xanthochromic, and may contain particles grossly visible to the eye. The number of white blood cells in the spinal fluid increases considerably, the usual range being between 300 and 700 per cu mm, while the number of cryptococci may become very great A cryptococcal count of the spinal fluid of one of our recent patients reached 8000 per cu mm The predominant cell type is the lymphocyte, although occasionally 95 per cent of the total may be polymorphonuclear leukocytes Spinal fluid albumin and globulin are in-

Institute of Pathology. The first evidence of complicating mycotic disease was noted when cryptococci were discovered in smears of bone marrow (Plate IIF). Subsequently the fungus was isolated and identified during life from the urine and spinal fluid, and at autopsy from the heart's blood



FIG 72 — The kidney is one of the organs most frequently involved in disseminated cryptococcosis *Although the fungus cells often become entrapped in glomerular capillaries, A, they* also pass into the tubules, *B* Hence urine culture is another important means by which a clinical diagnosis may be established *A* and *B* both are mucicarmine stains, × 95

1 CYTOLOGY OF THE FUNGUS

Cryptococcus neoformans in culture is a spherical to oval yeastlike cell which varies from 2 5 to 8 0 microns in diameter, but usually falls in the range of 4 to 7 microns[235 238] Occasionally in tissue the diameter, exclusive of the capsule, may reach 15 microns The cell reproduces by budding and is surrounded by a refractile mucinous capsule which is readily demonstrable in India ink mounts (Fig 73) Thickness of the capsule varies considerably with different strains and even within the same strain It may be very thin or it may reach 7 microns in thickness The encapsulated cell in culture has a total diameter which averages 10 microns but which may reach 18 microns Parent cells with single buds are always seen Some of our cultures have shown simultaneous

creased and, as a result, the specific gravity reaches the upper limit of the normal range (1.003–1.008). Sugar and chlorides are usually reduced, as occurs in tuberculous meningitis. The colloidal gold curve is variable, any type of reaction may be observed.

Disseminated infection by *C. neoformans seldom is recognized ante mortem* unless there are visible or otherwise demonstrable lesions in the skin, mucous

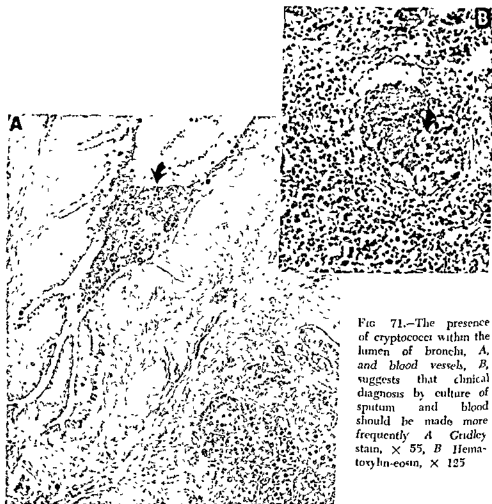


Fig 71.—The presence of cryptococci within the lumen of bronchi, *A*, *and blood* vessels, *B*, suggests that clinical diagnosis by culture of sputum and blood should be made more *frequently* *A* Gridley stain, × 55, *B* Hematoxylin-eosin, × 125

membranes, or bones Our pathologic studies, however, have revealed the frequency with which organisms may be found in the renal glomeruli and tubules (Fig 72), hepatic sinusoids (Fig 67), lymph nodes, and bone marrow (Plate II*E* and *F*) Such observations suggest that more frequent use of cultures of blood, urine, and tissues, obtained by needle or surgical biopsy, would establish the diagnosis more often As an example, we cite the case reported by Bowman and Ritchy,[38] in which *C neoformans* was recovered from the urine although repeated cultures of the blood and spinal fluid remained sterile. Another example is an unpublished case of Hodgkin's disease on file at the Armed Forces

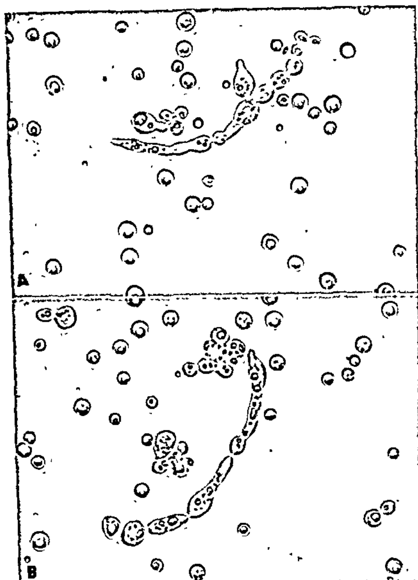


FIG 74 — *C neoformans* isolated from the spinal fluid of a 38-year-old white man who died of cryptococcal meningitis A rudimentary pseudomycelium is formed but there is no well developed mycelium The cytoplasm contains refractile granules and vacuoles, some of which are lipoidal (see Plate IIB). The cell wall is distinct and of moderate thickness Sabine mount of culture × 805 (AFIP Negative Nos. 55–10002 and 55–10003)

budding at several points on the mother cell[107] as well as formation of double buds,[83] and these usually have a thinner capsule than the parent cell (Fig. 73) No true mycelium is produced, but several cells in chains, or elongated yeastlike cells representing rudimentary pseudomycelium may be formed in culture (Fig 74) or tissues (Fig. 68). The cell wall is distinct and is of moderate thickness,

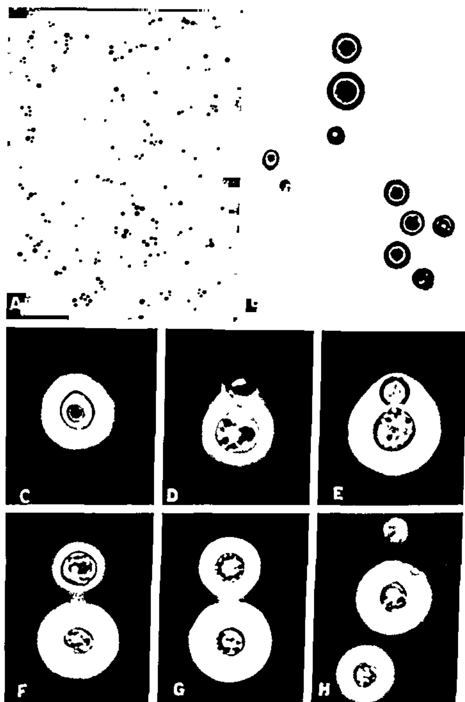


FIG 73 —India ink mount of spinal fluid sediment containing budding *C neoformans* *A* Mucinous capsule of the organism appears as a transparent halo, but not all cells are encapsulated × 100 *B* Reducing the light by lowering the substage condenser renders the central cell visible within its mucinous capsule × 805 *C* through *H* Budding process and formation of daughter cells × 1600 (AFIP Negative Nos 218120–477 to 481)

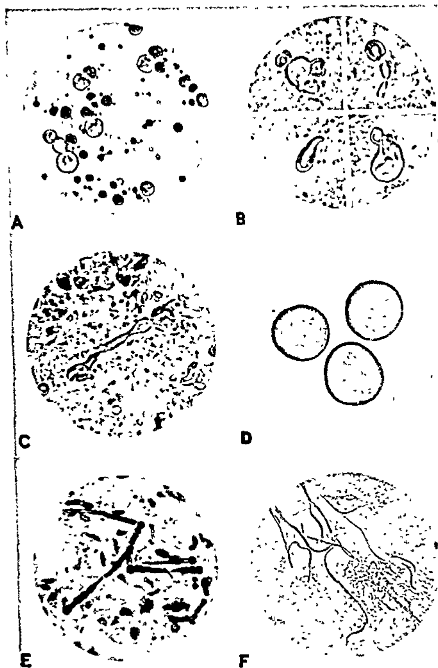


FIG 75—Artefacts in sputum described by Kurung[2] A Fat cell, presenting a superficial resemblance to budding yeasts × 800 *B* and *C* Colorless myelin globules also resemble budding forms and pseudomycelium × 800 *D* Maple pollen, × 800 *E* Asbestos bodies, which have a yellowish color × 800 *F* Highly refractile, slender, wavy fibrils of elastic tissue × 200 (Courtesy of J M Kurung[2])

but it is less conspicuous than that of yeast phase cells of *Blastomyces dermatitidis*. The cytoplasm usually contains refractile granules and vacuoles (Fig 73 and 74), some of which are lipoidal[135] (Plate IIB). Cells of pathogenic *C. neoformans* are more spherical and more highly granular than *C. neoformans* var. *innocuous*[26] Endospores are not produced, although some cultures do show a peculiar, thick-walled triangular cell containing a single body near the apex, interpreted by some as an ascospore[428] and by others as a modified capsule[25,387]

2. DIRECT EXAMINATION OF TISSUE FLUIDS AND EXUDATES

Because of its transparency, the capsule of the cryptococcus is not easily visualized under the microscope with ordinary illumination Reducing the light by lowering the substage condenser or by closing the iris diaphragm will facilitate its demonstration Specimens of pus, sputum, exudates and spinal fluid are first examined unstained with low and high power microscopy, and then by India ink mount. Sputum examination, in particular, should not be neglected, since demonstration of the organism and recovery in pure culture is relatively simple. Tenacious specimens are mixed in a test tube or on a slide, using equal volume of 10 per cent sodium or potassium hydroxide to dissolve tissue and blood elements and to free the fungal cells which resist the solvent effect of the alkali Specimens, if sufficiently fluid, may be mixed directly with fresh India ink and examined for encapsulated organisms Artefacts in sputum, occasionally confused with cryptococci and other fungi, are illustrated in Figure 75

Air-dried, and heat- or alcohol-fixed smears stained by Gram's method are completely unsatisfactory, and, in fact, may be misleading for the recognition of cryptococci in tissue fluids and exudates The extreme shrinkage and distortion of the fungus cells and the irregular staining occurring in such preparations (Fig 76) may cause the organisms to be overlooked or dismissed as crenated erythrocytes or disintegrated leukocytes

Spinal fluid is first examined without centrifugation If cryptococci are not detected on direct microscopic examination, the spinal fluid is then centrifuged with sterile precautions for ten minutes at 3,000 rpm, and the supernatant portion poured off for chemical studies

One drop of the sediment is cultured A second drop is mixed on a scrupulously clean slide with an equal volume of fresh undiluted India ink A cover glass is placed over the preparation immediately in order to prevent drying and the formation of artefacts It is first examined under low power (10X) objective of the microscope with lowered condenser (Fig 73A), then with high dry (43X) objective (Fig 73B) Oil immersion is unnecessary In the India ink mounts, the mucinous capsule of the cryptococcus appears as a large transparent halo Upon reducing the intensity of the light, the cell proper, with or without single buds, may be seen in the center of the capsule (Fig 73B) If bright illumination without India ink is used, the capsule is invisible, and the yeast cell may then be mistaken for a lymphocyte or a red blood cell

Demonstration of encapsulated yeastlike cells in spinal fluid is prima-facie evidence of cryptococcal meningitis, since no other encapsulated fungal species is capable of invading the nervous system of man or animals A second speci-

men, nevertheless, is always requested for further microscopic examination, culture and pathogenicity studies The persistence of "red blood cells" in the acetic acid diluent observed during the determination of spinal fluid cell counts should also serve as a clue to the presence of cryptococci. It is a disheartening experience to learn that pathogenic cryptococci have been mistaken for red blood cells and overlooked This pitfall may be avoided by using meticulous technic, sterile tubes, and a scrupulously clean counting chamber throughout spinal fluid collection and examination Any organism then demonstrated microscopically or by culture may be considered to be the responsible pathogen until definitely proved otherwise. Use also may be made of the metachromatic properties of the cryptococcal polysaccharide by adding 0 1 per cent toluidine blue to spinal fluid in the counting chamber. The cryptococcal cell becomes pink and the capsule is unstained, whereas white blood cells are deep blue, and erythrocytes remain unstained.[51]

Capsular "quellung" reaction permits a more precise identification of cryptococci than the mere demonstration of a capsule in India ink. The capsular reaction is demonstrated by mixing one drop of high titer type-specific anti-cryptococcal rabbit serum with equal volume of saline suspension of the culture, or with cryptococcus-laden spinal fluid itself, and examining microscopically under reduced light. The capsule of the organism will stand out sharply in a typical reaction with homologous type serum (Fig. 36), but not with serum-free normal saline or with heterologous type serum [114,296] However, cross reactions with *Candida albicans, Saccharomyces cerevisiae*, and other antigens have been reported [118]

In 1935, Benham was unable to make a serologic differentiation of pathogenic *C. neoformans* from *C. neoformans* var. *innocuous*. The reactions of the latter organism with the more recently developed type-specific antisera of *C neoformans*, serotypes A, B and C, have not yet been reported. The botanical classification of *C neoformans* is presented on pages 108–111

Since the normal skin is a recognized habitat for cryptococci,[331] finding encapsulated yeast cells on direct microscopic examination of exudate from cutaneous lesions does not constitute unequivocal evidence of cutaneous cryptococcosis, particularly if the organism is *C. neoformans* var *innocuous*. We have made it a general rule, when a strain of encapsulated yeast is recovered from any site in the body other than the central nervous system, to question its pathogenicity and identity as *C. neoformans* and to subject the culture to careful study (see Criteria for Identification of an Organism as *C neoformans*, page 118) When the answer is not definitive, biopsy of the suspected area may provide confirmatory evidence of invasion. This is potentially hazardous for the patient, however, unless the entire lesion is removed by block dissection, for theoretically biopsy could provoke dissemination of the organism

3 CULTURE STUDIES

C. neoformans grows on most of the common bacteriologic culture media and forms visible colonies within a few days, but develops much more abundantly when a carbohydrate such as dextrose (1–2 per cent) is included in the substrate (Fig 77). The colonial appearance and rate of growth varies with the



FIG 76 —Pure culture of *C neoformans* *A* Air-dried, heat-fixed and Gram stained, the shrinkage of the fungus cells and the irregular staining with crystal violet make this method of examination unsuitable for diagnosis The yeast cells could be mistaken easily for disintegrated leukocytes or crenated erythrocytes × 805 *B* Same culture in saline mount, although the cells appear several times larger, both preparations were photographed at the same magnification Pseudomycelium is present × 805 (AFIP Negative Nos 55–10001 and 55–10004, respectively)

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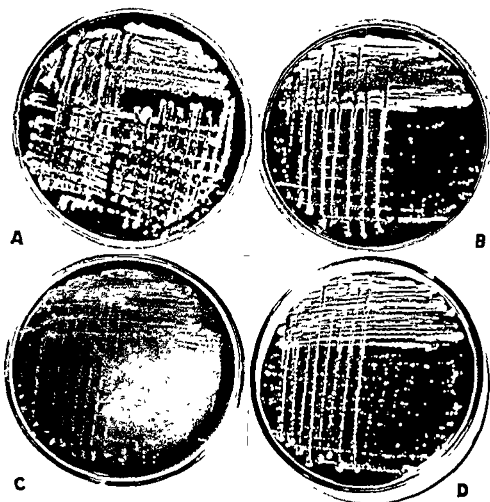


FIG 77 —*C neoformans* recovered from a fatal case of cryptococcal meningoencephalitis One loopful of culture was streaked across four different culture media and incubated for three days *A* Liver spleen glucose blood agar, 37°C *B* Littman oxgall agar, 20°C. *C* Brain-heart infusion blood agar, 37°C *D* Sabouraud dextrose agar, 37°C The low dextrose content (0 2 per cent) accounts for the scanty growth of *C neoformans* on brain-heart infusion blood agar, a culture medium employed widely in clinical laboratories Abundant growth occurs in culture media that contain 1 to 2 per cent carbohydrate, but Sabouraud dextrose agar containing 4 per cent dextrose does not promote the heaviest growth, presumably due to the absence of thiamine or other growth-promoting organic substances Liver-spleen glucose blood agar, containing 1 per cent dextrose, stimulates the heaviest growth[183]

culture medium used, and these are described on pages 101–103 Yeasts developing on culture media for tubercle bacilli should be examined carefully, since *C neoformans* survives alkali digestion[2] Thiamin, a specific requirement, enhances the growth and mucoid character of *C neoformans*.[338] An important cultural difference from other systemic fungi is that *C neoformans* forms smooth yeast colonies not only at 37°C but also at 20°C. Its cytologic characteristics also are the same at both temperatures, in contrast to diphasic (dimorphic) pathogenic fungi *Histoplasma capsulatum, Blastomyces dermatitidis, Blastomy-*

ces brasiliensis and *Sporotrichum schenckii* The latter reproduce as yeast phase in tissues and in culture at 37°C, but in culture at lower temperatures appear as filamentous molds with mycelium and spores

Cultures identical in all respects with *C. neoformans*, except in their production of minimal capsules and the formation of pasty, nonmucoid colonies, have been classified by Lodder and Kreger-Van Rij[218] as *C. neoformans* var. *uniguttulatus*. They studied two such strains isolated from human nails but did not report the pathogenicity of the organisms or tolerance to 37°C We have encountered in spinal fluid thinly encapsulated cryptococci which at first form pasty colonies on agar (Plate IIIC and F). However, these strains usually become well encapsulated upon intracerebral passage through mice, turn mucoid after prolonged incubation on dextrose agar media, and tolerate incubation at 37°C. We consider them to be typical *C. neoformans*.

One source of confusion in the identification of *C neoformans* is the presence of nonpathogenic encapsulated cryptococci in air, sputum, and skin Such strains, morphologically identical with *C. neoformans*, and referred to more properly as *C. neoformans* var. *innocuous*, have either feeble pathogenicity for mice or none at all, and are unable to grow at 37°C[26] Isolates from patients must be cultured at both 20°C and 37°C, in order to differentiate the pathogen from the nonpathogen

For incubation at 20°C, specimens are seeded on Sabouraud dextrose agar,* Mycological agar,* Mycophil agar,† all containing penicillin and streptomycin, or Littman oxgall agar* (Littman Medium)† (See Appendix formulas 1, 2, 3, 4 and 5) The latter medium is especially valuable for primary isolation of the fungus from pus, sputum, or other specimens heavily contaminated with fast growing saprophytic fungi and bacteria, since it limits spread of fungal colonies and restricts growth of bacteria[18,230,231,232]

For cultivation at 37°C, specimens are planted on brain-heart infusion blood agar medium and liver-spleen glucose blood agar medium (see Appendix formulas 6 and 7), both of which contain penicillin (20 units/ml) and streptomycin (50 units/ml) in order to inhibit the growth of contaminating bacteria The latter medium, used for incubation at 37°C, has a marked growth-stimulating effect upon pathogenic fungi, including *Cryptococcus neoformans* (Fig 77) It makes possible the recovery of yeast phase cells of *Histoplasma capsulatum* in numbers 3×10^4 times greater than with Sabouraud dextrose agar and other unenriched basal culture media[232]

For study of colonial morphology, cultures may be transplanted to Sabouraud dextrose agar, yeast morphology agar[163] or to malt-extract agar, and incubated at 37 and 20°C for several weeks (See Appendix, formulas 1 and 8)

Agar slant cultures of *C neoformans*, as well as other fungi, may be maintained in a viable state without drying for extended periods of time by covering the growth with sterile mineral oil and refrigerating at 5°C[2,44,232,321]

a *Sabouraud dextrose agar (20°C): C. neoformans* grows slowly on this medium, in opaque, yeastlike, creamy colonies which appear within three to

* Difco Laboratories, Detroit, Michigan, U S A

† Baltimore Biological Laboratory, Inc , Baltimore, Md , U S A

five days, and it produces a heavier growth than *C neoformans* var. *innocuous* [26] Colonies of *C. neoformans* are irregularly circular in shape, have a smooth, [illegible] (Plate III*D* and *E*). Microscopic Examination of an India ink mount [illegible] none at all (Fig. 73) A few cells form germ tubes, which represent an abortive effort on the part of the yeast to form pseudomycelium (Fig 74). Upon cultivation for another seven days, the density and opalescence of the colony give way to radial sectors of translucency, and it becomes mucoid, smooth, and assumes greater convexity. Microscopic preparations now show encapsulated budding yeast cells. After about ten days of incubation it flows down the agar plate or slant when it is maintained in the vertical position or even when maintained flat. Strains differ in color from light cream to orange-tan and brown but do not form carotenoid pigments as do *Rhodotorula* species

By seeding a large area (2 to 3 cm) of agar culture medium and incubating seven to fourteen days, characteristic giant colonies of the type illustrated in Plate III*D* to *G* are produced. A giant colony of *C. neoformans* on Sabouraud maltose agar may form sectors of three types mucoid, rough, or smooth.[96] Mucoid sectors are made up of cells possessing enormous capsules measuring up to 10 microns in thickness Smooth sectors are the most stable and have cells 4 to 6 microns in diameter, with thin capsules, while rough sectors have unencapsulated cells which tend to form pseudomycelium Maltose is reported to favor capsule formation more than dextrose [96]

b *Littman oxgall agar (20°C):* The organism grows more slowly than on Sabouraud dextrose agar Distinct colonies, measuring 4 to 6 mm. appear in four and six days respectively They are opaque, and at first have a bluish cast derived from the crystal violet in the medium, then they become grayish tan, butyrous in consistency, circular, with smooth, slightly raised surface and smooth edge (Plate III*A* and *B*) This culture medium is satisfactory only for incubation at 20°C or room temperature, and not 37°C Colonies on primary isolation superficially may resemble those of *Candida* species (Plate III*C*) Microscopic examination reveals oval, budding yeast cells with thin capsules, although a few well encapsulated cells may be found after diligent search On continued cultivation or successive transplantation, the colony develops radial sectors of pearly translucency (Plate III*B*), becomes mucoid, and, if incubation is prolonged in the inverted position for about 10 days, grows as a pointed cone, finally flowing onto the covering dish in the same manner as on Sabouraud dextrose agar The color of the colony deepens to light tan or brown Slide preparations now show encapsulated budding yeast cells

c *Brain-heart infusion blood agar (37°C):* The organism grows fairly rapidly, appearing within 48 to 72 hours as opaque, creamy, mucoid, raised circular colonies with smooth edges, somewhat resembling Friedlander's bacillus or mucoid Aerobacter Microscopic examination shows encapsulated budding yeastlike cells

d *Littman liver-spleen glucose blood agar* (37°C): The organisms grow very rapidly, appearing within 24 hours as colonies that are dense, opaque, creamy tan, butyrous in consistency, circular, with shiny raised surfaces and smooth edges (Plate IIIH and I) On continued cultivation, the color deepens rapidly to a dark tan and sectoring may occur (Plate IIII). Microscopic examination shows encapsulated budding yeastlike cells

e *Colony appearance of C. neoformans var. innocuous:* No growth takes place at 37°C On Sabouraud dextrose agar at 20°C, growth is less heavy and the colonies are more mucoid than *C. neoformans* On yeast morphology agar (Appendix, formula 8), colonies of *C. neoformans* var. *innocuous* are pale pink in color in three weeks at 20°C, while *C neoformans*, uncolored at first, assumes a deep, orange-tan color in the same period of time.[39]

f *Ascospore production:* The status of *C. neoformans* with regard to ascospore production is discussed under Historical Aspects, page 1, Cytology, of the Fungus, page 93, and Addendum, page 147. The ability to form ascospores is a most important characteristic for a yeast, since it serves as a means of classification. The most common mode of reproduction by yeasts is asexual, either by budding or fission, less frequently it is by sexual means Sexual reproduction occurs after the fusion of two yeast cells which form a short conjugation tube between them (isogamic and heterogamic conjugation) The nuclei of the two cells unite in the conjugation tube, and since cell division is accompanied by a reduction of chromosomes, the process is sexual Further nuclear division takes place to provide four or eight daughter nuclei, and spore walls form about each The large cell or sac is now called an *ascus*, and its internal spores, ascopores

The ability of a yeast to form *ascospores* is determined by cultivation at 25°C On culture media low in assimilable carbohydrates, such as Henrici's vegetable-juice sporulating agar (modified by Wickerham, Flickinger and Burton[465]) (see Appendix, formula 13), genera of *Hansenula* and *Pichia* form ascospores in three days, *Saccharomyces* in five to seven days, and *Debaryomyces* in five to twenty days. Other media satisfactory for sporulation are carrot agar (see Appendix, formula 14), malt extract agar, carrot plug, Gorodkowa agar, potato plug, and gypsum block

It is a relatively simple procedure to stain selectively for ascospores Air-dried, heat-fixed water suspensions of the growth on Henrici's vegetable juice sporulating agar are treated from one to three minutes with 5 per cent aqueous malachite green, gently warmed, water rinsed, alcohol decolorized, and counterstained with 0 5 per cent aqueous safranin[392] Ascospores stain green by this method, while the walls of asci and vegetative cells stain pink An alternate method of staining is by the ordinary Ziehl-Neelsen acid-fast technic, which colors ascopores bright red, and asci and vegetative cells blue However, spores of species of *Schizosaccharomyces* are not acid-fast[463]

We have not observed ascospore formation by any of our cultures of *C neoformans* or *C neoformans* var *innocuous* Lodder and Kreger-Van Rij[238] were

unable to demonstrate ascospores or cell conjugation in any of the 26 strains of *C. neoformans* they studied, and specific staining for ascospores was uniformly negative. Since various methods to induce spores failed, Lodder and Kreger-Van Rij[258] were unwilling to accept the phenomenon reported by Todd and Hermann[425] as ascospore development. In their recent classification, therefore, Lodder and Kreger-Van Rij retained the pathogenic species in the genus *Cryptococcus* (see also Addendum, page 147).

g. *Temperature tolerance and heat resistance: C. neoformans* grows abundantly over a wide range of temperatures, luxuriantly at 37°C and apparently best at 29°C.[224] *C. neoformans* var. *innocuous* and many other harmless species are unable to grow at 37°C. The ability to grow at this temperature is an important identifying characteristic for pathogenic *C. neoformans*, whose upper limit of heat tolerance is 39°C.

Because of the possible value of fever therapy as an adjunct in the treatment of cryptococcic meningitis, resistance to heat has been studied by numerous workers (see Hyperthermia, page 125). It is noted that the holding and flash methods of pasteurization exceed the maximal temperature tolerated by the organism and therefore destroy the fungus.

PLATE III COLONIAL CHARACTERISTICS OF *C NEOFORMANS* AND *C NEOFORMANS* VAR *INNOCUOUS*

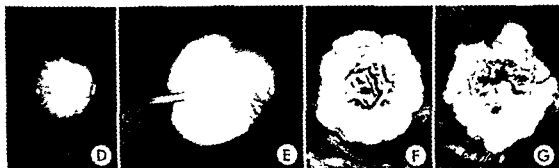
First row LITTMAN OXGALL AGAR (LITTMAN MEDIUM) *A*—*Cryptococcus neoformans*, giant colony incubated at 20°C for 7 days, growth is mucoid and sectoring has occurred, see page 102 and compare with Fig 77, actual size *B*—Same colony as *A* after 18 days incubation at 20°C, actual size *C*—*Cryptococcus neoformans*, pasty colony of the type often encountered on primary isolation, closely resembling that of *Candida albicans* It becomes mucoid on further subculturing Incubated at 20°C for 18 days, see page 101 and compare with *F*, actual size (AFIP Neg Nos 218120–C56, C105, C287)

Second row SABOURAUD DEXTROSE AGAR *D*—*Cryptococcus neoformans*, giant colony incubated at 20°C for 7 days, actual size The colony is smooth, glossy, raised and mucoid The radial sectors of translucency represent areas containing cells with wide capsules, see page 101 and Fig 77 *E*—Same colony illustrated in *D* after 18 days incubation at 20°C, actual size *F*—*Cryptococcus neoformans*, isolated from a patient with fatal cryptococcal meningitis, giant colony incubated at 37°C for 12 days, actual size Note pasty appearance of colony Compare with *C* and see also Fig 77 *G*—*Cryptococcus neoformans* var *innocuous*, giant colony incubated at 20°C for 18 days, actual size The mucoid colony is indistinguishable morphologically from that of *C neoformans*, but it would not develop at 37°C Compare with *E* and see page 103 (AFIP Neg Nos 218120–C103, C125, 580, 581)

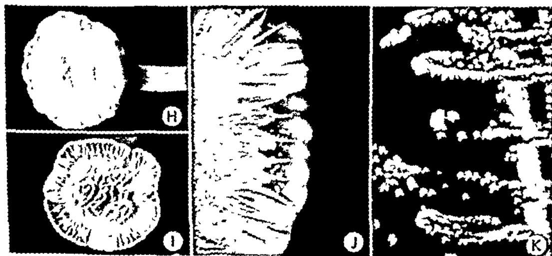
Third row LITTMAN LIVER-SPLEEN GLUCOSE BLOOD AGAR *H*—*Cryptococcus neoformans*, isolated from another fatal case of cryptococcal meningitis, giant colony incubated at 37°C for 8 days, actual size The raised mucoid colony produced flowing growth with the agar plate maintained flat *I*—*Cryptococcus neoformans*, same strain as in *F*, incubated at 37°C for 8 days, actual size Well defined sectoring has occurred, compare with growth on Sabouraud dextrose agar *F* and with different strain shown in *H* *J*—*Cryptococcus neoformans*, magnified colony illustrated in *I* Dense areas contain thinly encapsulated cells while translucent areas are composed of thickly encapsulated cells *K*—*Cryptococcus neoformans*, streak plate incubated at 37°C for 3 days Compare with giant colony of the same organism in *H* and with Fig 77 (AFIP Neg Nos 218120–582 to 585)



Littman Oxgall Agar (Littman Medium), see facing page



Sabouraud Dextrose Agar, see facing page.



Littman Liver-Spleen Glucose Blood Agar, see facing page

4 BIOCHEMICAL AND PHYSIOLOGIC REACTIONS

For taxonomic purposes there are several biochemical and physiologic properties of *C neoformans* which are especially important These include its methods of carbohydrate and nitrogen assimilation, its ability to produce starchlike compounds, and its requirement for oxygen.

A yeast either may assimilate a carbohydrate oxidatively, without appreciable change in the pH of the medium, or it may oxidize it incompletely in a "fermentation," with production of organic acids, alcohols and gases, and cause detectable pH changes. *C. neoformans* does not produce gas with any known carbohydrate and hence is not considered a fermentative* yeast, although it does produce acids from sugar. On the other hand, saprophytic yeasts are ready fermenters, producing both acid and gas—thus providing a convenient means for differentiation

Five soil strains of *C. neoformans*, three human strains, and the original strain of Sanfelice, which had been in Dunham fermentation tubes for three weeks at 30°C, all were reported by Emmons[107] to produce acid slowly in dextrose, levulose (fructose), mannose and sucrose, but not in glycerol or lactose. No gas was formed in any sugar Of 62 strains isolated by Pounden[324] from a severe outbreak of bovine mastitis, all produced acid with dextrose, levulose (fructose), and mannose, 50 produced acid in sucrose; 24 in maltose, 19 in starch, and none in galactose.[108]

Carbohydrates which are fermented are also assimilated, but the converse is not always true In the identification of *C. neoformans*, assimilation of carbohydrate rather than detection of fermentation has greater taxonomic value.

a *Carbon auxanographic agar method:* The carbon assimilation test introduced by Beijerinck[22] measures the ability of an organism to utilize a single carbohydrate (or carbon source) in a carbon-free synthetic medium (see Appendix, formula 9) Organisms capable of assimilating this carbohydrate will grow on the medium, otherwise, they will not develop at all The test is performed by seeding melted synthetic basal agar, free of carbohydrate, with the test organism, allowing the agar to solidify, and incubating for a few hours to dry the surface. Small quantities of dry carbohydrates are deposited on the surface of the agar, with dextrose as a control, since all yeasts assimilate this source of carbon No more than three or four carbohydrates should be tested at one time. After 48 hours incubation at 25°C, visible growth appears around those carbohydrates which the organism is capable of assimilating. In the event of uncertain results, the medium may be enriched by yeast extract or B vitamins and the culture retested, or it may be tested in a synthetic liquid medium (Appendix, formula 10) Twenty-six strains of *C neoformans* studied in this manner by Lodder and Kreger-Van Rij[236] were able to assimilate dextrose, maltose, sucrose and galactose, but not lactose These results were corroborated by Benham,[24] who tested 17 strains

* The term "fermentative" is employed in the mycologic sense to designate gas formation and not merely acid production without gas

b. *Carbon assimilation broth technic:* Wickerham and Burton[461] and Wickerham[463] objected to the agar plate method for assimilation tests on the basis of the inadequate basal medium, the short incubation period and the addition of yeast extract, since it supplied carbon and complicated reading of the results. They also considered an incubation period of 48 hours too short for successful use of pentoses, polyhydric alcohols and organic acids as test materials. To overcome these objections, a carbohydrate-free liquid medium was devised by them (Appendix, formula 10). Carbohydrate solutions to be tested are added aseptically to the basal medium to make a final concentration of 0 5 per cent, and tubes are seeded with one drop of yeast suspension. A similarly inoculated tube without carbohydrate, and a tube containing dextrose, serve as controls. Growth and turbidity result if the yeast is able to assimilate the test carbohydrate; otherwise the tube remains clear Incubation is at 25°C for seven and twenty-four days for "rapid" and "latent" reactions. Readings to determine density of growth are made with photoelectric cell or by placing against a white card bearing India-inked lines approximately 0 75 mm. thick and 5 mm. apart. If the lines are obliterated, growth is recorded as 3+; if they appear as diffuse bands, as 2+, if they are distinguishable but have indistinct edges, as 1+; if no growth, as negative. It was the author's opinion that assimilation tests would prove most valuable of all the biochemical procedures used in the classification of the yeasts. For *C. neoformans*, results obtained with the broth technic were essentially the same as those from the agar method, i e., assimilation of dextrose, maltose, sucrose, galactose but not of lactose [26]

c *Nitrogen assimilation:* The ability of yeasts to utilize peptone, ammonium sulfate, asparagine, urea and peptone when added to a nitrogen-free medium has been used as a means of classification In 1946, Wickerham[462] showed that in his liquid synthetic medium the presence of an adequate supply of vitamins permitted all the yeast strains tested to utilize these nitrogenous compounds separately, thus invalidating their use for classification. Lodder and Kreger-Van Rij[238] found the same held true for the nitrogen auxanographic agar method, however, this did not apply to utilization of nitrates as a sole source of nitrogen The ability of a yeast to assimilate nitrate is a stable characteristic on which many workers have differentiated genera and species of yeasts

d *Nitrogen auxanographic agar method.* The nitrogen assimilation test measures the ability of an organism to utilize a single nitrogenous compound added to a nitrogen-free synthetic medium (see Appendix, formula 11) Growth occurs only if the organism is able to assimilate the nitrogen in the compound being tested The technic is identical with that for testing assimilation of carbon except for the appropriate changes in culture medium Potassium nitrate is the only nitrogenous compound tested, while peptone serves as the control substance Agar plates are incubated at 25°C and read in 48 hours When tested in this manner, *C. neoformans* does not assimilate nitrate, while *C neoformans* var. *innocuous* does so readily.

e. *Nitrogen assimilation broth technic:* If results in the nitrogen auxanographic agar method are doubtful, a synthetic liquid test medium may be used (see Appendix, formula 12). Potassium nitrate, in final concentration of 0 078 per cent, is the only source of nitrogen tested. Ammonium sulfate in final concentration of 1 per cent, rather than peptone, serves as control substance in the liquid medium. The results with broth are essentially the same as with the agar technic, *C. neoformans* being unable to assimilate nitrate.

f. *Streak plate method for carbon and nitrogen assimilation test:* A new technic, somewhat simpler than the methods of Beijerinck and Wickerham, was described by Benham.[26] This consists of adding to Wickerham's yeast nitrogen base (Appendix formula 10) or yeast carbon base (Appendix, formula 12), as the case may be, a "purified" agar* containing KNO_3 or the carbohydrate to be tested The basal media are prepared in 10X strength with addition of either KNO_3 or carbohydrate to give the required final concentration and are sterilized by Seitz filtration. Two ml. sterilized solution are placed in a petri dish and 18 ml. melted and tempered, sterile 2 per cent "purified" agar are added, mixed thoroughly, and allowed to harden Plates are inoculated by streaking organ- [illegible]

first streak due to carry-over of nutrients with the inoculum, while the remaining streaks remained clear. Strains of virulent and non-virulent cryptococci gave consistently similar results with all three methods,[26] but streak plate and auxanographic agar were easier to prepare and gave clear-cut results in 48 hours In taxonomic studies where large numbers of compounds are tested and longer incubation periods are needed, the liquid media (Appendix formulas 10 and 12) were considered to be more advantageous.

g. *Production of starch-like compounds:* While studying biochemical properties of nonfermenting encapsulated yeasts, Aschner, Mager and Leibowitz[10] observed that *C. neoformans* produced extracellular starch when grown in a synthetic dextrose-thiamine agar at pH below 5 (see Appendix, formula 15). This observation was extended to 16 additional strains by Mager and Aschner[275] Non-pathogenic encapsulated yeasts capable of the same reaction were differentiated from *C neoformans* by failure to grow at 37°C Although the significance of starch production is not fully evaluated as yet, it has been considered sufficiently useful for taxonomic purposes by Lodder and Kreger-Van Rij[258] to assist in defining the genus *Cryptococcus*

After one or two weeks' incubation at 20°C on synthetic dextrose-thiamine agar the presence of starch-like compounds is made apparent by the addition

* Three types of purified agars all gave equally satisfactory results
(1) Washed agar described by Lodder and Kreger-Van Rij[258] (See Appendix, formula 9*a*).
(2) Agar purified by Robbins' method[366]
(3) Special agar, Noble, Difco Laboratories, Detroit, Michigan
"Washed agar," was recommended as the cheapest and easiest to prepare.

of Lugol's iodine, which turns the streak of growth blue. The extracellular character of the starch is demonstrated by the blue color of the agar substrate after the cellular growth is scraped away. The blue-staining product was identified as a polysaccharide of the amylose class by Hehre, Carlson and Hamilton.[175] All species of the genera *Cryptococcus* and *Bullera* formed extracellular starch, as did *Rhodotorula glutinis, Candida humicola, Candida curvata* and *Trichosporon cutaneum*.[258]

h. *Oxygen requirement, gelatin liquefaction, urease formation:* All strains of *C. neoformans* studied by Cox and Tolhurst[83] were reported to be aerobic, to liquefy gelatin to a slight degree in six to nine weeks and to turn litmus milk alkaline. Four of our strains of *C. neoformans* and five strains of *C. neoformans* var. *innocuous*[*] actively produced urease.

5 BOTANICAL CLASSIFICATION OF *C. NEOFORMANS* AND ASPOROGENOUS YEASTS

The terminology and classification adopted by Lodder and Kreger-Van Rij in their comprehensive 1952 publication, *The Yeasts, A Taxonomic Study*, has been accepted by American and European workers. Since asporogenous yeasts

TABLE III.—CLASSIFICATION OF THE ASPOROGENOUS YEASTS,[*] FAMILY *CRYPTOCOCCACEAE*
(*After Lodder and Kreger-Van Rij*[258])

Subfamily *Cryptococcoideae* (Arthrospores absent)	Subfamily *Trichosporoideae* (Arthrospores formed)	Subfamily *Rhodotoruloideae* (Pigment formed)
Genera	Genera	Genera
1 *Cryptococcus*[a]	1 *Trichosporon*	1 *Rhodotorula*[c]
2 *Torulopsis*[b]		
3 *Pityrosporum*		
4 *Brettanomyces*		
5 *Candida*		
6 *Kloeckera*		
7 *Trigonopsis*		

[*] Yeasts which form neither ascospores nor ballistospores, but which may form arthrospores.
[a] Formerly *Cryptococcus* Group II and III (Benham[33])
[b] Formerly *Cryptococcus* Group I (Benham[33])
[c] Formerly *Cryptococcus* Group IV (Benham[33])

such as *C. neoformans* do not form ascospores, they belong in the class of *Fungi imperfecti* or *Deuteromycetes*. Lodder and Kreger-Van Rij collected the asporogenous yeasts into a separate order, the *Cryptococcales*, and recognized one family in it, the *Cryptococcaceae*. In this family were placed both mycelial and non-mycelial asporogenous yeasts in three subdivisions, or subfamilies: *Cryptococcoideae, Trichosporoideae* and *Rhodotoruloideae* (See Table III, above). The genus *Cryptococcus*, placed with the subfamily *Cryptococcoideae*,

[*] Obtained through the courtesy of Dr. R. W. Benham, Columbia University, New York.

TABLE IV.—DIFFERENTIAL CHARACTERISTICS OF GENERA OF ASPOROGENOUS YEASTS IN FAMILY *CRYPTOCOCCACEAE*
(*After Lodder and Kreger-Van Rij*[164])

Subfamily *Cryptococcoideae*	
1. *Cryptococcus*	Spherical or ovoid cells, budding, rudimentary pseudomycelium, encapsulated, extracellular starch produced, no ascospores, nonfermenter (7 species*)
2 *Torulopsis*	Spherical or ovoid cells, budding, no extracellular starch, rarely encapsulated, active fermenter (22 species, one variety)
3 *Pityrosporum*	Ovoid or bottle-shaped cells, budding, not encapsulated, no pseudomycelium or mycelium, difficult to cultivate on ordinary laboratory media, nonfermenter (2 species)
4 *Brettanomyces*	Ovoid, spherical or elongate cells, budding, primitive pseudomycelium, strong acid production, active fermenter (4 species, 2 varieties)
5 *Candida*	Cells of varying shape, budding, chlamydospores, blastospores, true mycelium or well-developed pseudomycelium, fermenter (30 species, 6 varieties)
6 *Kloeckera*	Lemon-shaped or sausage-shaped cells, bipolar budding, primitive pseudomycelium, strong fermenter Nitrate not assimilated (8 species)
7 *Trigonopsis*	Triangular or ellipsoidal cells, budding at angles and multilaterally, no pseudomycelium, no fermentation (one species)
Subfamily *Trichosporoideae*	
1 *Trichosporon*	Cells of varying shape, chlamydospores, blastospores, pseudomycelium or true mycelium, arthrospores (8 species, one variety)
Subfamily *Rhodotoruloideae*	
1 *Rhodotorula*	Cells round, oval or elongate, budding, primitive pseudomycelium, distinct red or yellow carotenoid pigments, nonfermenter (7 species, one variety)

* Five species of Lodder and Kreger-Van Rij plus 2 species added by Benham [26]

was limited to spherical or oval encapsulated cells which reproduce by multilateral budding, do not produce a true mycelium, but may form a rudimentary pseudomycelium (Fig 74), do not produce ascospores, are nonfermenters and produce extracellular starch under special conditions On the basis of the size and shape of cells and carbon and nitrogen assimilation, Lodder and Kreger-Van Rij recognized five species, *C neoformans*, *C laurentii*, *C albidus*, *C luteolus* and *C diffluens* However, this classification was made on purely taxonomic grounds without pathogenicity studies in animals In a re-evaluation of the criteria for the identification of species of cryptococci, Benham utilized virulence tests She studied Lodder and Kreger-Van Rij's 5 species and in addition, strains of *C neoformans* var *innocuous* and *C mucorugosus* (Table V, page 110) No truly virulent species other than *C neoformans* was encountered in the genus *Cryptococcus* She confirmed the usefulness of nonassimilation of nitrates as a valuable criterion for identification and recommended its addition to other diagnostic features of *C neoformans*, such as its ability to grow at 37°C and its virulence for mice Carbohydrate assimilation tests were found to be of value in differentiating species (see Table V, page 110) As a result of her studies, Benham[26] listed 2 additional species in the genus *Cryptococcus*, namely *C. neoformans* var *innocuous* and *C. mucorugosus* Some strains of *C neoformans*

var. *innocuous* were found to be identical or closely related to Lodder and Kreger-Van Rij's *C. albidus*, *C. diffluens* and *C. luteolus* (see Table V).

TABLE V—DIFFERENTIATION OF GENUS *CRYPTOCOCCUS* KÜTZING EMEND VUILLEMIN
(*After Lodder and Kreger-Van Rij*,[158] *Benham*[26])

Organism	Growth at 37°C	Pathogenicity for mice	KNO_3 Assimilation	Carbon assimilation: Dextrose	Maltose	Sucrose	Lactose	Galactose	Capsule	Extracellular Starch
C. neoformans	+	+	0	+	+	+	0	+	+	+
C. laurentii	0*	0†	0	+	+	+	+	+	+	+
C. luteolus	0*	0†	0	+	+	+	0	+	+	+
C. mucorugosus‡	0*	0†	+	+	+	+	+	+	+	+
C. albidus§ *C. diffluens*§ *C. neoformans* var. *innocuous*‡	0	0	+	+	+	+	0	0	+	+

* Sometimes weak growth at 37°C.
† Sometimes mild degree of virulence, recoverable from mouse peritoneal cavity but never invades brain.
‡ Benham's species.
§ *C. albidus* reported to assimilate lactose and galactose and *C. diffluens* to assimilate galactose.[258]

Synonymy. Since *Cryptococcus neoformans* (Sanfelice) Vuillemin, 1901 has been isolated by many investigators from widely differing vegetable, animal and human sources in different regions of the world, an imposing list of synonyms has accumulated.[149] By eliminating fermenting yeasts and those reported to produce mycelium, characteristics which are incompatible with *C. neoformans*, Lodder and Kreger-Van Rij[259] narrowed this list to 39 valid synonyms, as follows:

Saccharomyces neoformans Sanfelice (1895)[illegible]
Torula neoformans (Sanfelice) Weis (1902)[illegible]
Blastomyces neoformans (Sanf.) Arzt* (1924)[illegible]
Torulopsis neoformans (Sanf.) Redaelli (1931)[illegible]
Debaryomyces neoformans (Sanf.) Red., Cif. et Giordano (1937)[illegible]
Saccharomyces lithogenes Sanfelice (1895)[illegible]
Cryptococcus lithogenes (Sanf.) Vuillemin (1901)[illegible]
Blastomyces lithogenes (Sanf.) Sasakawa (1922)[illegible]

* Although this synonym is often found in the literature, it was not Arzt who named the organism he described, but probably Benedek, who sent this strain to the Centraalbureau voor Schimmelcultures at Baarn, Holland, labeled in this manner.

Torulopsis lithogenes (Sanf) de Almeida (1933)
Cryptococcus hominis (Vuillemin) (1901)
Atelosaccharomyces hominis (Vuill) Verdun (1912)
Torulopsis hominis (Vuill) Redaelli (1931)
Debaryomyces hominis (Vuill) Todd et Herrmann (1936)
Saccharomyces hominis Costantin (1901)
Cryptococcus costantini Froilano de Mello et Gonzaga Fernandes (1918)
Torulopsis costantini (Froilano de Mello et Gonzaga Fernandes) de Almeida (1933)
Saccharomyces plimmeri Costantin (1901)
Torula plimmeri (Costantin) Weis (1902)
Cryptococcus plimmeri (Costantin) Neveu-Lemaire (1912)
Torulopsis plimmeri (Costantin) de Almeida (1933)
Torula kleini Weis (1902)
Cryptococcus kleini (Weis) Cohn (1904) apud Guéguen
Atelosaccharomyces busse-buschki de Beurmann et Gougerot (1909)
Saccharomyces blanchardi Guiart (1910)
Atelosaccharomyces breweri Verdun (1912)
Cryptococcus breweri (Verdun) Castellani et Chalmers (1913)
Saccharomyces breweri (Verdun) Neveu-Lemaire (1921)
Torulopsis breweri (Verdun) de Almeida (1933)
Torula histolytica Stoddard et Cutler (1916)
Torulopsis histolytica (Stoddard et Cutler) Castellani et Jacono (1933)
Cryptococcus cerebriloculosus Freeman et Weidman (1923)
Torula nasalis Harrison (1928)
Cryptococcus nasalis (Harrison) C W Dodge (1935)
Cryptococcus hondurianus Castellani, Castellani et Jacono (1933)
Cryptococcus hominis Vuill var *hondurianus* Castellani, Castellani et Jacono (1933)
Torulopsis hominis (Vuill) Red var *hondurianus* Castellani, Castellani et Jacono (1933)
Cryptococcus psicrophylicus Niño (1934)
Torulopsis neoformans (Sanf) Red var *sheppei* Giordano (1935)
Cryptococcus meningitidis C W Dodge (1935)

A nonencapsulated, fermenting yeast, formerly classified as *Cryptococcus glabrata* but now placed in another genus as *Torulopsis glabrata* (see Table IV, page 109), has been isolated infrequently but exclusively from human sources, i e, pharynx, sputum, urine, uterus, cervix, fallopian tube and feces [5,36,318] In Plaut's patient the organism was present in endometrial curettings over a period of two years, and sections of cervix, endometrium and fallopian tubes contained similar organisms within areas of granulomatous inflammation Benham identified the organism as *C glabrata* Recent studies by Lopez Fernández[282] in Montevideo have shown that in the experimental animal *T glabrata* infection simulates histoplasmosis The organisms appear as small intracytoplasmic parasites and there is an intense productive reaction of the reticuloendothelial system The authors suggest that some of the human cases of histoplasmosis diagnosed by histopathologic methods could represent *T glabrata* infection.

6 PATHOGENICITY, VIRULENCE FOR ANIMALS

The only pathogenic species of the genus *Cryptococcus* is *C neoformans* The virulence of this organism is unaltered by growth on culture media but seems to vary with different strains [25] Serial passage through white Swiss mice increases its virulence, but this enhancement is of a lower order than usually encountered with bacterial or viral pathogens similarly treated While white

Swiss mice and rats exhibit definite individual variations in susceptibility to infection, they are the most susceptible of all animal species. Guinea pigs are less prone to infection, while rabbits are relatively resistant. *C. neoformans* var. *innocuous*, morphologically identical and culturally similar to *C. neoformans*, is differentiated by (1) its failure to grow at 37°C, (2) its nonvirulence and (3) by biochemical differences (see Table V, page 110).

a. *White Swiss mice:* The white Swiss mouse is the ideal animal for experimental work in cryptococcosis. The most effective method for demonstrating pathogenicity of *C. neoformans* is by intracerebral injection of 0.02 to 0.04 ml suspension of the organism in saline into 6 week old white Swiss mice. Cryptococcus-laden spinal fluid may also be used. The injection is made with a tuberculin syringe and 26 gauge ¼" (5 mm.) needle through the skull into the cerebral cortex with the animal under light ether anesthesia. Injection is made with a rotating movement of the needle into the posterior half of the skull, about one-quarter of an inch lateral to the midline in order to avoid the superior saggital sinus. Smith, Mosberg and Manganiello[391] described a similar technic of inserting a 26 gauge needle 3 mm. in length, through the squamosal suture, midway between the eye and the ear, and injecting 0.1 ml. suspension of cryptococci.

Practically all strains of *C. neoformans* derived from spinal fluid, when injected intracerebrally into white Swiss mice, will produce a lethal effect in five to fourteen days. Several days following injection the mice become quiescent, their coats roughen, and they huddle together in a corner of the cage. Around the fifth day frank cerebellar symptoms appear and the mice stagger about in circles. Because of the softness and thinness of the calvarium of young mice, the increased intracranial pressure that results from cerebral infection with cryptococci produces marked hydrocephalus, and the occipital region of the skull bulges prominently (Fig. 78), although this is not a uniform occurrence. When the mouse is sacrificed, its brain is found to be edematous and hyperemic. The encapsulated yeast is demonstrated readily by touching the surface of the brain with a platinum loop and preparing an India ink mount with this material. Pathologic examination reveals the cerebral matter to be studded with pinpoint cysts laden with encapsulated cryptococci. The susceptibility of the white Swiss mouse to intracerebral injection of *C. neoformans* and its resistance to biologically related microorganisms is demonstrated by Emmons' experiment with intracerebral injections of ordinary yeasts and *Candida* suspensions which failed to produce a lethal effect.[108] Emmons was unable to find living organisms in the brains of the animals one month after injection.

Injection of white Swiss mice intravenously with *C. neoformans* via the tail vein using a 27 gauge needle will kill the animals within 10 to 16 days. Multiple lesions are formed in the lungs, kidneys, spleen, brain and liver (Fig. 79).

Intraperitoneal injection, on the other hand, is not as uniformly lethal as the intracerebral or intravenous routes. Of twelve white Swiss mice injected intraperitoneally with 8 million cryptococci by Kligman and Weidman,[220] only nine died within the experimental period, with involvement of the lungs, kidneys, brain, spleen and liver. Cox and Tolhurst[63] found that mice which survived three weeks or longer following intraperitoneal injection with *C. neoformans*

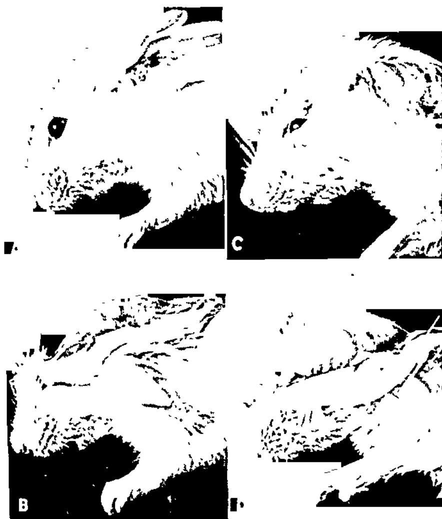


FIG 78 —Intracerebral injection of white Swiss mice with *C neoformans* produces neurologic symptoms, marked hydrocephalus and death in five to fourteen days The prominent bulging of the cranium is a characteristic feature of intracerebral inoculation *A* and *B* illustrate contour of normal mouse head *C* and *D* show pronounced occipital bulging in mouse five days after intracerebral injection of 0 02 ml suspension of *C. neoformans* (AFIP Negative No 218120—586 to 590)

showed multiple macroscopic tumor-like masses in the liver, spleen, kidneys, lymph nodes, and other viscera Subcutaneous and intranasal inoculation failed to kill a significant number of mice Wade and Stevenson[441] were unable to produce lesions of the nervous system of white mice by subcutaneous, intranasal, or intratracheal inoculation

b *Rat: C neoformans* is less pathogenic for rats than for white Swiss mice. Since the skull of the rat is relatively thick, intracerebral injection must be made

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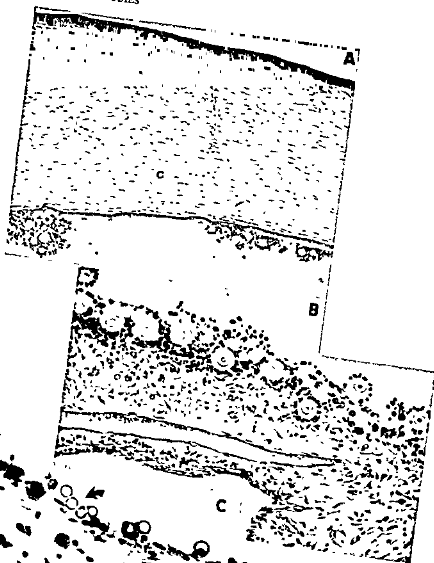


Fig 80—*A* and *B* Experimental injection of cryptococci into anterior chamber of rabbit eye by Weiss, Perry and Shevky [89] (AFIP Accession No 624829, contributed by Dr I Perry) Clumps of inflammatory cells, among which are seen thickly encapsulated cryptococci, are present on the inner surface of the cornea (c) in *A*, and along the anterior surface of the iris (i) in *B* *C* Russell bodies, sometimes mistaken for fungus cells are seen in the stroma and on the anterior surface of the iris (arrows) The basophilic staining and lack of a capsule are typical features which differentiate the Russell body from cryptococci (AFIP Accession No 207045, contributed by Dr F H Verhoeff) *A* × 80 *B* × 150 *C* × 450, all hematoxylin and eosin



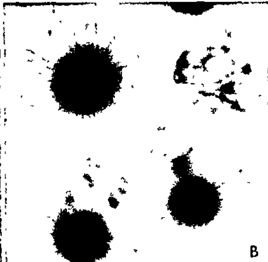
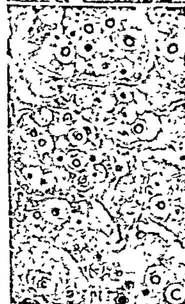
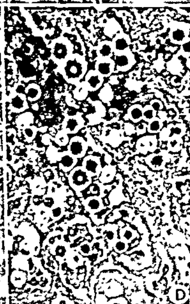
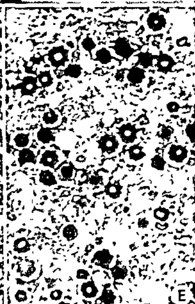
Fig 79—Cryptococcal nodules and miliary lesions in the lungs of a white Swiss mouse, fourteen days after intravenous injection with *C neoformans*. (AFIP Negative No 218120–569)

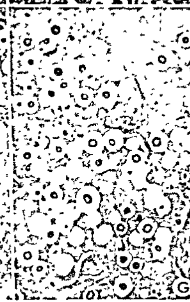
after trephination, with the animal under anesthesia. Intraperitoneal injection, however, is lethal for a majority of rats in from four to twenty-four days, although some animals may survive for months. Of eighteen rats injected by intraperitoneal, intrapleural and intracardiac routes, sixteen subsequently developed lesions in the brain, lungs, liver, spleen, kidney and lymph nodes.[407]

c *Guinea pig:* Many workers have utilized guinea pigs for the determination of strain pathogenicity and have reported results ranging from no effect at all to a lethal effect in six weeks.

d. *Rabbit:* This species tolerates injections of *C. neoformans* by various routes without harm, although some strains of cryptococci have produced generalized infection and death. The resistance of the rabbit to *C. neoformans* has been attributed to its high normal temperature of 103°F (39 5°C)[224, 391] This *native resistance, together with the well defined heat susceptibility of the organism* (Table I, page 46), formed the basis for considering hyperpyrexia in the therapy of human cryptococcosis The average body temperature of other small animals is listed in Table VII (page 127). In spite of the rabbit's resistance to systemic infection, the anterior chamber of the rabbit eye appears quite susceptible to *C neoformans*[220, 457] One-tenth ml of a diluted 24 hour culture is injected into the anterior chamber of one eye, with the rabbit under intravenous pentobarbital and ether anesthesia Care is exercised not to injure the iris Keratitis and iritis are visible within one week after injection, reaching full development within fourteen to seventeen days Section of the enucleated eye reveals characteristic lesions along the anterior surface of the iris and posterior surface of the cornea (Fig 80) These consist of a festoon of rosettes, each composed of a single encapsulated cryptococcus cell, ringed by inflammatory cells

e. *Chick embryo:* Nine day old chick embryos are highly susceptible to intravenous injection of 0 03 ml suspension of *C neoformans* which contains


A

B

C

D

E

F

G

H

approximately 600,000 cells;[220] the authors believe that this technic may have promise in the evaluation of chemotherapeutic agents. By adjusting the size of the inoculum, Kligman, Crane and Norris,[215] were able to produce almost 100 per cent mortality in the embryos within nine days following injection The organisms were seen in great numbers in the liver, spleen, brain, kidneys and lungs of the dead embryos. Inflammatory reaction was minimal, and there were no granulomas or abscesses. Surface inoculation of the chorioallantoic membrane did not have a lethal effect.[220]

7. HISTOPATHOLOGIC DIAGNOSIS

Fungus infections lend themselves to recognition and identification in tissue by histopathologic technics more readily than those caused by bacteria or viruses. This is due to the characteristic tissue reaction which they produce and to the fact that fungus cells often are easily detected in tissues because of their relatively large size and, with few exceptions, their ready staining by conventional and special technics. This is particularly true of *Cryptococcus neoformans*, whose large cells and great numbers in the lesions of man and animals permit undelayed diagnosis of the mycotic nature of the disease.

Identification of C. *neoformans* in tissues is based on the following observations:

(a) The fungus is a spherical to ovoid budding, yeastlike cell that varies greatly in size but generally has a diameter of 5 to 10 microns, exclusive of the capsule. The cytoplasm stains light pink with hematoxylin and eosin, sometimes faint gray-blue, but never the deep blue of yeast phase cells of *Blastomyces dermatitidis* and *Coccidioides immitis*

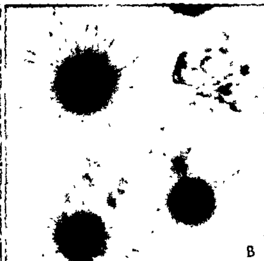
(b) The cell wall is distinct, delicate and slightly acidophilic. It is sometimes anisotropic and may appear thickened or doubly contoured. When so modified, it resembles that of yeast phase *Blastomyces dermatitidis.*

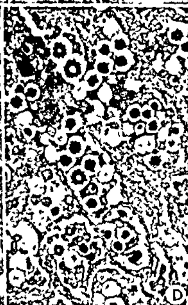
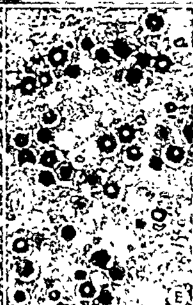
(c) The cryptococcus has a characteristic mucinous capsule, measuring 0 5 to 5 times the diameter of the cell proper, which ordinarily fails to stain by the usual histologic methods and therefore causes the cell to appear with an unstained halo about it

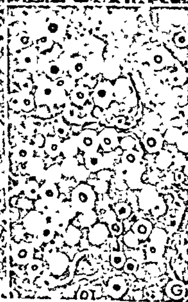
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PLATE IV ENCAPSULATED CRYPTOCOCCI DEMONSTRATED BY SPECIAL STAINING PROCEDURES

A—Hematoxylin eosin, × 150
B—Toluidine blue, frozen section, AFIP Accession No 337884, × 1450
C—Mucicarmine, × 150
D—Periodic acid-Schiff, × 150
E—Acid mucopolysaccharide, × 150
F—Bauer stain, × 150
G—Gridley fungus stain, × 150
H—Gridley reticulum stain, × 150
Fields shown in *A, C, D, E, F, G* and *H* represent adjacent sections of lung (AFIP Accession No 557547) The capsular mucopolysaccharide, barely visible in *A, F, G* and *H*, is seen readily in *B, C, D* and *E* This demonstrates that the familiar halo observed about the *Cryptococcus* cell in routine histologic preparations is, in fact, capsular material and is not caused artificially by shrinkage of the cell during fixation All sections illustrated were prepared from formalin-fixed tissue and all except *B* were embedded in paraffin


A

B

C

D

E

F

G

H

(d) Histochemical studies* (Plate IV) reveal the presence of a mucopolysaccharide in the capsule and cell wall of *C. neoformans* demonstrable by the mucicarmine stain for mucin[245] and the Rhinehart-Abul-Haj technic for acid mucopolysaccharides[244] This is of diagnostic importance since, to our knowledge, no other yeastlike fungus† of similar morphology stains in this distinctive manner While other special histochemical methods, e g Hotchkiss-McManus (periodic acid-Schiff),[197,217,273] Bauer,[19] Gridley,[157] and Gomori,[151] are excellent for demonstrating the cryptococcus cell, they stain other fungi equally well. Gram staining reveals cryptococci to be Gram-positive but this is less specific and less valuable as a diagnostic method.

(e) *Reproduction of cryptococci in tissue is by budding* Thin-walled cellular projections, round to oval in shape and of varying sizes, are seen attached to parent cells and single daughter cells are found detached. Chains of three or four cells are occasionally seen but rarely more than this number (Fig. 68). So-called "germ-tubes" or elongated fingerlike projections which represent rudimentary pseudomycelium are seen in tissues, but no true mycelium or any specialized fungal structures such as sporangia or endospores are developed by this fungus

Although the usual fatal case of cryptococcosis is recognized readily by the histopathologist and diagnosed accurately, it is the localized infections encountered in surgical pathology that prove to be the most troublesome diagnostically Difficulties may be encountered when the organisms are very few in number and are obscured by inflammatory cells, when they stain poorly, and when they are mistaken for other budding fungal species because of atypical morphologic features In such instances, special staining technics prove to be most valuable and should be employed routinely Thus the Gridley stain[157] is especially valuable for the detection of small numbers of organisms buried among a host of inflammatory cells The Bauer stain[19] and Hotchkiss-McManus method[197,217,273] will also stain fungi in tissues brilliantly and are extremely useful for the same purpose In localized infections, cryptococci may be small, thinly encapsulated and when engulfed by giant cells and histiocytes may resemble *Histoplasma capsulatum* Mayer's mucicarmine and Best's carmine stains[245] readily differentiate the two organisms, since neither *Histoplasma* nor other fungi† possesses the pink to red-staining mucinous capsular material of *Cryptococcus neoformans*

With the general increase of interest in mycology and the awareness by pathologists that mycotic diseases are more common than was appreciated formerly, the pendulum has begun to swing in the direction of overdiagnosis We have seen cases in consultation and found reports in the literature in which a diagnosis of one or another of the mycoses has been based on artefacts in

* See Appendix, formulas 16–20, for useful histochemical procedures

† The mature segmented spores contained within the large sporangium of *Rhinosporidium seeberi*, the cause of rhinosporidiosis, are coated with a mucicarminophilic matrix In animal tissue the cigar-shaped spores of Sporotrichum *schenckii may* also be enveloped in a mucicarmine-positive material Because of the obvious morphologic dissimilarities of these two organisms to *C neoformans*, their differentiation *should not offer* difficulties

tissue sections. *All sorts of cellular elements and tissue debris may be misinterpreted.* However, the most important of these are mineral concretions, Russell bodies, PAS-positive granules of tissue origin, phagocytosed nuclear debris and hydropic inflammatory cells with pyknotic nuclei. Some of these are illustrated in Figures 80–82. *A safe rule is that if the diagnosis of cryptococcosis is considered and a thorough search of representative sections through all portions of the lesion in question fails to yield typical and unequivocal encapsulated cryptococci, then another etiology must be sought.* In our experience it is unnecessary to base a pathologic diagnosis of cryptococcosis upon the presence of but a few atypical or otherwise questionable fungus cells, providing

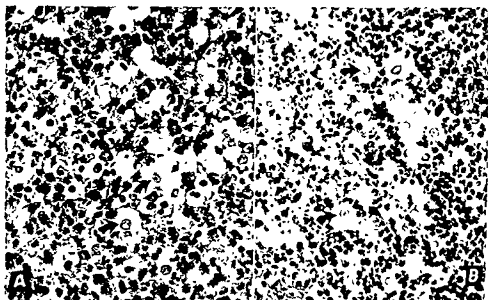


FIG 81 —*A* Extremely hydropic inflammatory cells with pyknotic or karyolytic nuclei may be misinterpreted as cryptococci The cells in *A* (arrow), stained with hematoxylin-eosin, were remarkably similar to cryptococci, but stains for fungi and for mucin were consistently negative. *From a case of probable allergic granulomatosis of the nose* (*AFIP Accession No* 697708, contributed by Veterans Administration Hospital, Martinsburg, West Virginia) × 410 *B* From a fatal case of cryptococcal meningoencephalitis Cells shown in *B* (same stain) appear almost identical with those in *A*, but gave typical histochemical reactions of cryptococci (AFIP Accession No 265063) × 290

adequate material, properly stained, is available for study. Densely calcified lesions ascribed to cryptococcosis are reported from time to time. We, on the other hand, have been impressed by the rarity with which significant calcification is observed in this infection, and are therefore highly skeptical of those *reports in which all of the fungi had become transformed into calcified fossils.*



Fig 82.—A and B Mineral concretions which may present an extremely disturbing similarity to budding fungus cells The possibility that fungus cells may become calcified in tissue cannot be denied, but it seems quite unlikely that such degenerative changes would occur in actively dividing cells The absence of the characteristic cryptococcal capsule is especially helpful in excluding the diagnosis of cryptococcosis A Hematoxylin-eosin, × 300 (AFIP Negative No 55-8519) B Hematoxylin-eosin × 410 (AFIP negative No 55-8518) Thick walled Russell bodies presenting a "double contour," are shown in C (arrows), a more characteristic cluster of Russell bodies is also present to right of center Hematoxylin-eosin, × 610 (AFIP Accession No 78171)

8. CRITERIA FOR IDENTIFICATION OF AN ORGANISM AS *C NEOFORMANS*

a *Cellular morphology:* Spherical or oval encapsulated yeast cell, 4 to 7 microns in diameter averaging 10 microns with capsule, sometimes reaching 18 microns. Capsule thickness varies from faint shell to 7 microns. Reproduces by budding with single buds, occasionally by double budding No mycelium formed, but culture may have fragments of rudimentary pseudomycelium Identical cytologic features at 20°C and 37°C. Gram-positive No arthrospores or ascospores.

b *Colony formation:* Rapid growth on dextrose agar to form tan, pasty colony which becomes mucoid and flows over the agar. Sectoring occurs

c. *Oxygen requirement:* Aerobic, oxidative.

d *Tolerance to 37°C:* Grows rapidly at 37°C (*C. neoformans* var *innocuous* and other cryptococcal species do not grow at this temperature.)

e. *Biochemical:* No gas production from any sugars, acid production from sugars variable, forms extracellular starch from dextrose-thiamine agar. Capable of growth in synthetic medium with the following carbohydrates as a sole source of carbon dextrose, maltose, sucrose, galactose but not lactose. Incapable of growth in a synthetic medium with KNO_3 as sole source of nitrogen (*C. neoformans* var *innocuous* can assimilate KNO_3, dextrose, maltose, sucrose but not galactose or lactose).

f. *Serologic:* Serotypes A, B and C on basis of specific capsular polysaccharide Type-specific capsular reaction.

g *Pathogenicity:* Intracerebral inoculation lethal to white Swiss mice in five to fourteen days, intravenous injection lethal in ten to sixteen days (*C neoformans* var *innocuous* is non-pathogenic)

h. *Histopathologic.* Encapsulated budding cells showing considerable variation in size, shape and staining characteristics, no mycelium or specialized structures, rarely pseudomycelium In common with most fungi, stains positively with Gram's stain and with Gomori, Gridley, Bauer and Hotchkiss-McManus (periodic acid-Schiff) technics Differentiated from other yeastlike fungi by mucicarmine-positive capsule containing demonstrable acid mucopolysaccharide

X. Treatment and Prognosis

Cryptococcosis is often considered to be synonymous with meningoencephalitis This is due to the relatively great neurotropism of the fungus and to the fact that, until recent years, the infection was recognized only after the nervous system had become involved. It is unfortunate that cryptococcus infection should be thought of only in association with nervous system disease, since this affects adversely the opportunity for early diagnosis, effective treatment, and cure Prognosis is poor once the fungus has spread to the brain and meninges Approximately 80 to 90 per cent of patients with meningoencephalitis die within the first year, and many within six months of the onset of neurologic manifesta-

cures are extremely rare, if obtained at all. The fact that periods of remission may occur with spontaneous disappearance of organisms from the spinal fluid must be constantly borne in mind during the clinical evaluation of new fungicidal drugs An exceedingly long period of follow-up is essential before "cures" can be claimed

1 SURGICAL TREATMENT OF LOCALIZED LESIONS

Despite the poor prognosis of patients with meningoencephalitis, there is reason for optimism With the realization that most cases of nervous system cryptococcosis probably represent complications of subclinical pulmonary infection, an ever increasing number of cases are being reported in which spread to the brain has been prevented by surgical resection of the involved lung. For this reason, emphasis must now be placed on early diagnosis of obscure pulmonary infections If the lesion can be detected early and removed completely before hematogenous spread, the prognosis is excellent An optimistic outlook is taken in Australia, where several patients, in whom nervous system involvement had already occurred, nonetheless underwent surgical resection of localized pulmonary granulomas with encouraging results

a *Pulmonary*. The most important, and certainly the most encouraging aspect of the treatment of cryptococcosis is the current practice of surgically extirpating localized pulmonary lesions Such lesions have a number of charac-

many patients may be asymptomatic when the lesion is detected by routine chest x-ray examination, (d) delay in surgical intervention or inadequate resection with incision into the infected lung tissue may deny the patient a potential cure

Surgical extirpation of localized pulmonary lesions, as advocated by Taber[116]

in 1937, has been reported with increasing frequency during the past decade Probably the first well documented "cure" by pulmonary resection was that reported by Froio and Bailey[138] in 1949. Their patient, a 19 year old U.S Army inductee, was asymptomatic at the time a routine chest x-ray examination revealed a localized mass in the right lower lobe. Bronchogenic carcinoma was suspected. At operation, a well circumscribed firm mass of rubbery consistency, gray-white in color and measuring 4 5 cm. in diameter, was present in the lower lobe, and pneumonectomy was performed When this case was first published in 1949, the patient's progress had been followed for over five years, and later Haugen and Baker[172] reported the same patient to be well eight years after operation. Starr and Geddes[402] described the case of a 21 year old Australian soldier with a cryptococcal granuloma removed by lobectomy. Susman[411] reported this patient to be alive and well five years later. Such long follow-up periods are essential in order to assess properly the results of surgical treatment of cryptococcosis

An excellent example of the importance of prolonged follow-up is illustrated by the first case reported by Palmrose and Losh[314]* A 23 year old man had roentgenographic evidence of a mass in the right lower lobe This was enucleated at operation by blunt dissection and pathologic examination revealed it to be a cryptococcal granuloma The patient remained in the hospital for over a year because of postoperative complications (bronchopleural fistula and staphylococcal empyema) for which a 4-stage thoracoplasty was performed. More than two years later (three and one-half years after the original lung lesion had been enucleated) the patient was readmitted with cryptococcal meningoencephalitis, for which he received streptomycin, penicillin, Actidione, KI, and sulfadiazine, but he died in less than five months This case might well have been considered a "cure" two or three years after the original operation

One wonders, therefore, about the ultimate outcome in some of the surgically resected cases of pulmonary cryptococcosis, published after only short periods of observation[14, 320] Berk and Gerstl,[30] for example, reported a four-year cure in the case of a 30 year old veteran with asthma of three years' duration The patient had been found to have a localized, dense opacity in the left lung He subsequently developed a pleural effusion and low grade fever which did not respond to penicillin and sulfadiazine A left lower lobectomy was performed, and on frozen section a diagnosis of mycotic granuloma was established The lesion measured 7 × 6 × 6 cm Good results could be anticipated in this case since the lesion apparently was removed in its entirety with the excised lobe In the case of Palmrose and Losh cited previously, the mass merely was enucleated The question can be asked whether a more "radical" procedure might not have prevented the postoperative complications as well as the ultimate spread of infection to the nervous system The cases reported by Dormer and his associates[90, 91] furnish additional evidence as to the probable danger of incision or puncture of cryptococcal granulomas In both of their cases the pulmonary lesions had been "needled" and then partially excised Significantly, signs of meningitis appeared in both patients within a few weeks after operation

* This case bears AFIP Accession No 168877

A number of cases treated surgically have been reported from Australia, where cryptococcosis is either more prevalent or better recognized. It is certainly more vigerously treated there than in many other parts of the world. Pulmonary lesions are resected, not only to prevent nervous system involvement, but also in the hope that resection will have a beneficial effect in patients in whom meningoencephalitis is already established. Included in three cases ... that of a ... rovement ... Although recovery seemed to have occurred, the follow-up period was only six months. Susman[411] reported a similar case, also from Australia, in which a 43 year old man with systemic and meningitic cryptococcosis underwent pneumonectomy. He recovered and was back at work eleven months after operation Although reports from Australia are encouraging, longer periods of observation are necessary for confirmation of cure, nevertheless, they represent one bright spot in the otherwise dismal picture of the treatment of nervous system cryptococcosis.

Another question to be considered is, what happens to patients with solitary pulmonary lesions of cryptococcosis in whom resection is not performed? Spontaneous regression may occur,[246] but no matter how the disease process behaves roentgenographically, threat of dissemination is ever present All that can be said with assurance then is that the localized cryptococcal lesions are treacherous and unpredictable Swanson and Smith[412] reported the case of a man rejected for military service because of a large area of consolidation in the right upper lung field The area of involvement increased in size at first but then receded The sputum contained cryptococci Over a period of many months, during which the patient remained asymptomatic, the pulmonary infiltration disappeared roentgenographically Cerebellar symptoms appeared nonetheless, and the patient died Lesions were found at autopsy in the brain, lung, and spleen A similar sequence of events occurred in the case reported by Hamilton and Tyler[164] A woman patient* who had a negative chest x-ray eight months earlier developed pleuritic pain and roentgenographic evidence of a parenchymal lesion in the left lower lung During the ensuing seven months there was marked radiographic regression of the lesion, and the patient became symptom-free She remained asymptomatic an additional half year when manifestations of cryptococcal meningitis appeared Death occurred seven weeks later, although the pulmonary infiltration had almost disappeared by the time symptoms of meningitis appeared

In both of these cases, relatively long asymptomatic intervals had elapsed before nervous system involvement became evident, yet in other cases meningitis had developed soon after the discovery of an asymptomatic pulmonary lesion Susman's second case was that of a symptom-free 60 year old man who was discovered to have a radio-opaque lesion at the base of the right upper lobe[411] Several weeks later meningitis occurred and the patient died In such cases there may be no significant change in the radiologic aspects of the pulmonary mass Serial x-ray films of the lungs in the case reported by Ratcliffe

* This case bears AFIP Accession No 107139

and Cook*[129] revealed a remarkably stationary process. The patient, asymptomatic at the time he was a candidate for military service when his first x-ray examination was made, complained of headache only after two and one-half months of observation, and died of cryptococcal meningitis within four weeks

b. *Extrapulmonary:* Spread of cryptococcus infection from the lungs to other tissues is not always accompanied by diffuse meningoencephalitis or widespread dissemination. The infection may be localized in one area, where it then may become amenable to surgical extirpation. Even multiple extrapulmonary sites of localization have been excised with favorable results. One of the most radically treated cases in which good results were obtained was described by Kramer and associates [223] Their patient, a 32 year old Sikh, had a painful swelling over the left eye. Incision and drainage yielded a blood-stained, gelatinous exudate The lesion recurred and epileptiform seizures developed Roentgenographic evidence of erosion of the orbital walls and involvement of the frontal sinus was noted Several months later a fluctuant mass measuring 3×5 cm appeared over the right femoral trochanter, and incision produced viscid fluid containing cryptococci. Masses of granulation tissue were resected from the orbit and frontal and ethmoid sinuses. Several months later the intracranial extension of the infection was resected from the frontal lobe Histologic preparations disclosed cryptococci in tissues removed from the hip, orbit, sinuses, and brain Examination eleven months after the frontal lobe resection and more than two years after onset revealed the patient to be in good condition The only neurologic signs present were exaggerated patellar and Achilles reflexes on the right side This case is certainly most unusual, for seldom are neurosurgical procedures attended by such good results in cryptococcosis

Carton and Mount,[60] in their analysis of 178 cases of cryptococcosis involving the nervous system, found that symptoms indicating an expanding intracranial or intraspinal lesion were present in 23 per cent In almost all such cases, however, there is widespread meningoencephalitis in addition to the localized expanding lesion Thus, surgical extirpation of localized intracranial lesions ordinarily can be expected to provide only temporary improvement Ramamurthi and Anguli,[326] however, reported the successful resection of a cryptococcal granuloma of the spinal cord in a 17 year old Indian girl who had pain in the back, difficulty in walking, and exaggerated peripheral reflexes, but normal spinal fluid A year after operation she was well with sterile spinal fluid and normal chest x-ray films No antibiotics or chemotherapeutic agents had been administered

A number of other cases are on record in which infection was controlled by limited or radical surgical procedures Evacuation of the contents of localized cryptococcal "abscesses" and resection of diseased tissues apparently was sufficient to eliminate the infection involving the spine and paravertebral soft tissues in the case reported by Brewer and Wood[39] (one-year follow-up) and the pelvic and inguinal tissues in the case of McGehee and Michelson[267] (eight-month follow-up) Jones[206] reported a patient with extensive nasopharyngeal and palatine cryptococcosis cured by a combination of repeated excisions,

* This case bears AFIP Acc No 268527

cauterization, iodides, and x-ray therapy (two-year follow-up). The case reported by Kessel and Holtzwart[109] is of special interest because, after amputation of the leg for cryptococcal infection of the knee joint, two cutaneous lesions of the chest appeared and were healed by x-ray therapy.

Another case of localized cryptococcosis treated radically was reported by Burger and Morton[47] Their patient, a 41 year old woman, had a rapidly enlarging soft tissue mass in the thigh Preoperative diagnosis was established by aspiration which produced a thick, glairy, mucinous material in which organisms were discovered The operation consisted of amputation by disarticulation through the hip, and four and one-half years later the patient was alive and well Much less radical surgery is apparently sufficient in the smaller, more localized [illegible] reported by Dienst[71] and the one illustrated in Figs 22 [illegible] cryptococcosis cured by unilateral [illegible] ates[45]

2. NONSURGICAL [illegible]

A review of the literature reveals an evergrowing list of therapeutic agents, used largely without success. It includes a number of physical methods as well as chemical and biologic preparations, which are summarized in Table VI. Since it is well established that spontaneous remissions can occur in the course of cryptococcal meningitis, caution must be exercised in the evaluation of any particular method of therapy. An ideal anticryptococcal drug would have certain properties It would (1) be fungicidal, (2) reach sites of infection in effective concentration, (3) be nontoxic for man and for experimental animals, (4) eradicate infection in the experimental animal, and (5) effect prompt and permanent clinical remission consistently Rarely are all these criteria satisfied by any given therapeutic agent *An agent which seems to be effective in vitro sometimes is surprisingly ineffective in vivo, and vice versa Although effective concentrations may not be reached in normal tissues, therapeutic levels may be attained as a result of pathologic changes Dosage schedules which are therapeutically effective and nontoxic for an experimental animal may be either toxic or ineffective in man In evaluating animal experiments, mere prolongation of life is insufficient, for it must also be demonstrated that the infection has been eradicated*

a *Physical Agents:* The main physical agents that have been employed in the treatment of cryptococcosis are heat and roentgen rays

Hyperthermia C *neoformans* will not withstand temperature above 39°C (Table I, page 46) This fact, together with the observations that the rabbit, whose normal body temperature exceeds that of the mouse, rat, cat, dog, and guinea pig (Table VII),[391] is relatively resistant to cryptococcus infection,[25 224 225] suggested the use of fever therapy as an adjunct to medicinal agents in the treatment of human cryptococcosis Some supportive experimental evidence is also available C *neoformans* infection of chick embryos was controlled by incubation for 8 days at 104°F (40°C)[215] It has also been suggested that the effectiveness of Actidione in vitro is enhanced at elevated temperatures[379] Clinical evidence of the beneficial therapeutic effect of hyperthermia has also

been reported. Kligman and Weidman,[220] in treating a patient with both cryptococcal meningitis and malaria, observed that the meningeal symptoms abated during the paroxysms of fever and organisms disappeared from the spinal fluid. Carton[58] noted clinical improvement in a patient with cryptococcal meningitis following an attack of severe non-mycotic epididymitis which had produced a fever of 105°F for several hours. In evaluating these and other clinical observations, one must bear in mind the occurrence of spontaneous remissions.

In general, prolonged hyperthermia has not proved to be very useful in the treatment of human cryptococcosis. Hyperthermia by means of typhoid-paratyphoid vaccine was attempted by Cox and Tolhurst[83] in one of their cases of

TABLE VI.—SUMMARY OF NONSURGICAL THERAPY OF CRYPTOCOCCOSIS

A. *Physical Agents*

Fever therapy
- Heat cabinet[58, 76, 83, 254, 291]
- Typhoid-paratyphoid vaccine[83, 254, 291]

Ultra violet radiation[206]

X-ray therapy[58, 68, 76, 87, 155, 206, 210, 409, 450]

B. *Chemical Agents*

Heavy Metals
- Antimony compounds[284]
- Antimony and potassium tartrate[126]
- Arsenicals
  - Arsenic (Fowler's solution)[278]
  - Oxophenarsine hydrochloride[284]
  - Tryparsamide[68]
- Colloidal copper[83]
- Colloidal silver[378]
- Gold sodium thiosulfate[409]

Sulfonamides
- Azosulfamide[337]
- Gantrisin[287]
- Sulfadiazine[21, 30, 68, 72, 73, 78, 93, 124, 126, 151, 155, 163, 170, 207, 239, 254, 279, 289, 291, 309, 329, 339, 426, 439, 470]
- Sulfamerazine[76, 470]
- Sulfanilamide[279, 291, 339]
- Sulfapyridine[68, 239, 318, 337, 339, 357]
- Sulfathiazole[21, 126, 155, 162, 164, 170, 309, 345, 357, 426]

Diamidines
- Stilbamidine[287]
- 2-hydroxystilbamidine[461]

Other Agents
- Acriflavine[80, 142, 302, 450]
- Alcohol, intravenous[254]
- Alkalinization therapy[291]
- Atabrine[151]
- Calcium gluconate[291]
- Daraprim®[492]
- Diazone[291]
- Ethyl vanillate[247]
- Fuadin® (Stibophen)[21]
- Furacin®[291]
- Gentian violet[73, 155, 207, 309, 409, 470]
- Hexamethylenamine[409]
- Hexamine[63]
- Iodides, sodium and potassium[21, 47, 60, 66, 68, 76, 93, 124, 126, 142, 155, 164, 206, 207, 233, 239, 254, 285, 287, 279, 281, 291, 329, 333, 337, 339, 378, 412, 431, 439, 470]
- Lipiodal (40% iodine)[91]
- M & B 693[83, 239]
- Methylene blue[291]
- Para-amino-salicylic acid[163]
- Pregl's iodine solution[66]
- Promin[170]
- Pyribenzamine (Tripelennamine)[470]
- Sodium bicarbonate[124, 470]
- Sodium thiosulfate[83]
- Thiamin[291]
- Thymol[47, 207]

C. *Antibiotics*

- Actidione® (Cycloheximide)[11, 53, 58, 65, 101, 124, 141, 166, 170, 185, 186, 238, 254, 383, 413, 470]
- Aureomycin[163, 470]
- Chloromycetin[7, 470]
- Circulin[423]
- Nystatin[490] (Mycostatin)®
- Penicillin[30, 66, 72, 76, 121, 151, 155, 163, 161, 165, 170, 239, 254, 287, 289, 291, 309, 345, 347, 469, 470]
- Polymyxin[254, 329]
- Prodigiosin[21, 347]
- Protoanemonin[58, 60, 76]
- Streptomycin[58, 66, 72, 76, 124, 144, 163, 291, 329, 347, 470]
- Terramycin®[58, 163]
- Tyrocidin[155, 126]

D. *Immunologic Agents*

- Autogenous vaccine[21, 31, 60, 66, 83, 287, 409, 337, 378, 412]
- Gamma globulin[254]
- Immune rabbit serum[378]

E. *Enzymes*

- Hyaluronidase (Wydase®, Alidase)[254]
- Streptodornase[254]

cryptococcal meningitis, but a satisfactory elevation of body temperature was not maintained Collins[16] used hyperthermia, iodides and x-ray therapy in his Case 1, but the patient failed to improve and finally died. Mosberg and Arnold[291] gave typhoid vaccine to a patient with cryptococcal meningitis (their Case 3); as a result the following temperatures were achieved: 71 hours over 100°F, 40.5 hours, over 102°F, and 10 hours, over 104°F. No clinical improvement was obtained. In the same paper the authors reported another case in which the patient had to be removed from a heat cabinet after attaining a body temperature of 107.8°F within fifty minutes. The patient eventually died of the disease. Carton's Case 1 was kept in a heat cabinet for forty-three hours at 103°F, and for seven hours at 105.8°F, but had to be removed because of electrocardiographic changes attributed to hyperthermia. In Littman

TABLE VII.—NORMAL BODY TEMPERATURES OF EXPERIMENTAL ANIMALS
(*Modified from Smith et al*[393])

Animal	Average Body Temperature
Mouse	99.1°F
Rat	100°F
Dog	100.8°F
Cat	101°F
Guinea Pig	102.2°F
Rabbit	103.2°F
Pigeon	103°F

and Nathanson's[254] case of cryptococcal meningitis, intravenous typhoid-paratyphoid vaccine (4.5 × 10⁹ cells daily) produced temperatures of 103 to 104.5°F for nine consecutive days, but there was no clinical improvement and the patient died In all cases cited, a miscellany of chemotherapeutic, antibiotic, and other therapeutic agents was employed in combination with fever therapy For instance, Littman and Nathanson's patient received Actidione, polymyxin, hyaluronidase, streptodornase, iodides, sulfadiazine, penicillin and gamma [illegible] ...erthermia and alkalinization was [illegible][290] on the basis of in vitro studies [illegible] ...formans by increased pH. For example, only forty-eight hours' incubation (40°C) was required to kill the fungus in a synthetic mineral broth at pH 8.7, while 120 hours was necessary when the pH was 7.3 However, three patients with cryptococcal meningitis treated with oral and intravenous sodium bicarbonate, as well as typhoid vaccine, iodides, penicillin, streptomycin and sulfadiazine, eventually died of the disease[291] In Case 3 of Mosberg and Arnold,[291] the spinal fluid pH increased from 7.0 to 7.85 In our opinion, this mild degree of alkalinity would not retard the growth of *C neoformans*, for contrary to the popular belief that acidity of the culture medium favors the growth of pathogenic fungi, including cryptococci, and that alkalinity restricts it, the optimal pH for most pathogenic fungi lies in the neutral or slightly alkaline range[250] *C. neoformans* produces luxuriant growth at pH 7.7 in the presence of organic substrates (see Culture Studies and Appendix, Formula 7[252]) While the microorganism may be more sensitive to heat at higher pH values in a mineral broth,[290] this may not be true in the presence of protective organic substances in blood or spinal fluid.

been reported Kligman and Weidman,[220] in treating a patient with both cryptococcal meningitis and malaria, observed that the meningeal symptoms abated during the paroxysms of fever and organisms disappeared from the spinal fluid Carton[58] noted clinical improvement in a patient with cryptococcal meningitis following an attack of severe non-mycotic epididymitis which had produced a fever of 105°F for several hours In evaluating these and other clinical observations, one must bear in mind the occurrence of spontaneous remissions

In general, prolonged hyperthermia has not proved to be very useful in the treatment of human cryptococcosis Hyperthermia by means of typhoid-paratyphoid vaccine was attempted by Cox and Tolhurst[83] in one of their cases of

TABLE VI —SUMMARY OF NONSURGICAL THERAPY OF CRYPTOCOCCOSIS

A *Physical Agents*

- Fever therapy
  - Heat cabinet[58 76 83 254 291]
  - Typhoid-paratyphoid vaccine[83 254 291]
- Ultra violet radiation[206]
- X-ray therapy[58 66 76 87 155 206 210 409 450]

B *Chemical Agents*

- Heavy Metals
  - Antimony compounds[284]
  - Antimony and potassium tartrate[126]
  - Arsenicals
    - Arsenic (Fowler's solution)[378]
    - Oxophenarsine hydrochloride[254]
    - Tryparsamide[58]
  - Colloidal copper[83]
  - Colloidal silver[378]
  - Gold sodium thiosulfate[409]
- Sulfonamides
  - Azosulfamide[337]
  - Gantrisin[287]
  - Sulfadiazine[21 30 66 72 73 76 93 124 126 151 155 163 170 207 239 254 279 289 291 309 329 339 426 439 470]
  - Sulfamerazine[76 170]
  - Sulfanilamide[279 291 339]
  - Sulfapyridine[68 239 318 337 339 357]
  - Sulfathiazole[21 126 155 162 164 170 209 345 357 426]
- Diamidines
  - Stilbamidine[287]
  - 2-hydroxystilbamidine[461]
- Other Agents
  - Acriflavine[60 142 302 450]
  - Alcohol, intravenous[284]
  - Alkalinization therapy[291]
  - Atabrine[151]
  - Calcium gluconate[291]
  - *Daraprim*®[492]
  - Diazone[291]
  - Ethyl vanillate[247]
  - Fuadin® (Stibophen)[21]
  - Furacin®[291]
  - Gentian violet[72 155,207 309 409,470]
  - Hexamethylenamine[409]
  - Hexamine[65]
  - Iodides, sodium and potassium[21,47,60 66 68 76 93 124 128 142,155,161 206 207,223 239 254 265 277, 279 284 291 329 333 337,339 378 412 421 429 470]
  - Lipiodal (40% iodine)[91]
  - M & B 693[83 239]
  - Methylene blue[284]
  - Para-amino-salicylic acid[163]
  - Pregl's iodine solution[66]
  - Promin[170]
  - Pyribenzamine (Tripelennamine)[470]
  - Sodium bicarbonate[124 470]
  - Sodium thiosulfate[63]
  - Thiamin[291]
  - Thymol[47 207]

C *Antibiotics*

- Actidione® (Cycloheximide)[11 53 58 85 101 124, 141 156 170 185 186 238 254 383 413 470]
- Aureomycin[163 470]
- Chloromycetin®[470]
- Circulin[423]
- *Nystatin*[490] (*Mycostatin*)®
- Penicillin[30 66 72 76 121 151 155 163 161 165 170 239 254, 287,289,291 309 345 347 459 470]
- Polymyxin[254 329]
- Prodigiosin[21 347]
- Protoanemonin[58 60 76]
- Streptomycin[58 66 72 76 124 144 163 291 329 317 470]
- Terramycin®[58 163]
- Tyrocidin[155 426]

D *Immunologic Agents*

- Autogenous vaccine[21 31 60 66 81 287 309 337 376 412]
- Gamma globulin[254]
- Immune rabbit *serum*[376]

E *Enzymes*

- Hyaluronidase (Wydase®, Alidase)[254]
- Streptodornase[254]

effects in some patients with mycotic disease, improvement is often only temporary. In other instances, however, iodides have deleterious effects Patients hypersensitive to the invading fungus either did not improve or became worse rapidly when iodides were administered.[219] This was due to the liberation of the fungus and its products from the granulomatous tissue, necessitating a prior partial desensitization with *Blastomyces* vaccine. Iodides have been administered by several routes to patients with cryptococcal meningitis generally without beneficial effect (see Table VI, page 126)

Antimonials: Several antimony compounds, including antimony potassium tartrate and Fuadin* (stibophen), have been used without effect for treatment [illegible] meningitis[21,128,251] The failure of antimony potassium tartrate [illegible] to inhibit *C neoformans* was re- [illegible] Grunberg and Schnitzer[159] [illegible]

carbamyl] benzene [illegible]

pound, designated Ro-2-3094, was effective against [illegible] tions of 0 5 to 1 5 mg /ml or 500 to 1500 μg/ml (1500 μg/ml).[59] Ro-2-3094 compound was relatively nontoxic, the LD_{50} dose in mice being 350 mg /Kg intravenously.[158] Carton and Liebig[59] determined this dose to be 590 mg /Kg. Compound Ro-2-3094 was administered subcutaneously to 170 mice infected intraperitoneally with cryptococci, in a dosage of 50 mg /Kg daily for 21 days. There were 130 survivors, while in an untreated group of 108 infected mice [illegible] were only 22 survivors Autopsy of the treated mice revealed tissue ne- [illegible] results appear promising, the route [illegible]

effective for demonstrating [illegible]

therape[illegible] for Animals, page [illegible] Ne- [illegible]

theless, [illegible] nd further animal testing and clinical trial are warranted

Sulfonamides: In vitro studies by Fisher[134] revealed *C neoformans* to be relatively resistant to sulfonamides For example, 50 to 250 mg. per cent† sulfamerazine were required to produce "weak inhibition" Sulfadiazine in concentrations of 25, 250 and 500 mg per cent resulted in "weak," "slight" and [illegible] Kligman and Weidman[220] noted that con- [illegible] 400 concentration) of the following thirteen [illegible] *neoformans* in vitro sulfamerazine, sulfadi- [illegible] sulfanilamide, sodium-5-sulfanilamido-naphthalene-[illegible] fanilamido benzoate, [illegible] nilanilide, N[1]-p-nitrobenzoyl sulfanilamide, [illegible] and sodium bismuth mercaptoacetyl sulfathiazole Keeney, Ajello and Lankford[208] reported that 1000 mg per cent sulfadiazine was required to inhibit *C neoformans* completely Neither sulfathiazole nor sulfamerazine caused complete inhibition at this concentration, and the presence of whole blood re-

* Hoffmann-LaRoche, Inc , Nutley, N J

† One mg per cent equals 10 μg/ml

As a form of therapy, fever imposes a heavy burden on a patient already severely ill Induced elevation of body temperature by means of a heat cabinet is resisted at first by profuse diaphoresis until the body's heat regulating mechanisms are disturbed. Temperature rise may then be uncontrollable, as in one of Mosberg and Arnold's[291] cases, or it may produce irreversible electrocardiographic changes, as in Carton's[58] case. There is no convincing evidence at the present time that heat therapy is effective in the treatment of cryptococcal meningitis.

X-irradiation: Radiation therapy of cryptococcis has been reported infrequently. Favorable results have been obtained, however, in a few localized lesions. Chapman, cited by Stone and Sturdivant,[409] observed an inhibitory effect of x-rays upon the growth of *C neoformans* in broth culture and in spinal fluid.

Jones's[206] case of extensive nasopharyngeal and palatine cryptococcal infection was treated unsuccessfully with excision, cautery of the bases, and iodides. Following the institution of x-irradiation therapy, however, the nodules disappeared and further cauterization was unnecessary (two-year follow-up) Soft x-rays had been directed into the mouth as follows: one-fourth erythema dose, 6 inch gap, 5 milliamperes, no filter at 20 inch distance. The same procedure was repeated three times in a period of six weeks.

Two draining cutaneous lesions of the thorax of a patient were healed by high voltage x-ray therapy following amputation of the leg for infection of the knee joint[210] Two patients with pulmonary cryptococcosis have been given deep x-ray therapy[87,155] One with localized pulmonary consolidation received 450r directed to the lower lung fields posteriorly over a period of seventeen days At the end of the treatment the area of consolidation had decreased in size.[155] The other patient who received deep x-ray therapy to the chest had a cerebellar granuloma, he derived no benefit from the treatment[87] Although improvement has been noted in x-irradiated localized lesions, infection of the central nervous system appears to be uninfluenced by this form of treatment[68,409,450]

b *Chemotherapy:* Among the chemotherapeutic drugs that have been employed in cryptococcosis are iodides, a variety of metallic compounds, sulfonamides, diamidines, and many nonspecific agents (*Table VI*)

Iodides. Sodium and potassium iodides permeate extracellular fluids but exert little specific action on microorganisms Growth of *Candida albicans,* for example, is unchecked in the presence of 2 5 per cent (25000 μg/ml) potassium iodide[487] Iodides usually are administered to cause degeneration of granulomatous tissue When used in the treatment of late syphilitic lesions, iodides are said to remove indurated and nodular granulomas and to promote healing of ulcers[310]

Potassium iodide was used successfully in the treatment of cutaneous North American blastomycosis, but it has not proved to be curative for the pulmonary or systemic forms of the disease The more highly specific diamidine drugs, such as stilbamidine and 2-hydroxystilbamidine, have mostly replaced iodide therapy

Although administration of iodides is accompanied *by beneficial clinical*

tions.[6,37,75,212,367,377,392,393,395] When administered too rapidly by the intravenous route, stilbamidine may cause circulatory collapse. It is largely retained by the tissues and only 5 to 10 per cent is excreted in the urine.[395] The drug is concentrated in the liver, kidneys, adrenal and sweat glands, with the result that degenerative changes may be produced in these organs. Serious hepatic and renal disease may be contraindications, therefore, to the administration of stilbamidine. The drug also becomes concentrated in the nervous system, but the specific tissue varies in different animal species. In dogs it is the cerebral gray matter, while in man it is the peripheral nerves[107,395] The drug is unstable to light. Total doses exceeding 2 5 Gm are likely to cause trigeminal neuropathy in man, especially on exposure of the patient to sunlight.[393] Because of these and other objectionable features, closely related analogues of stilbamidine were prepared and tested

In the course of studies with 2-hydroxystilbamidine, Snapper[393] observed that it relieved the pain of multiple myeloma as effectively as did stilbamidine,

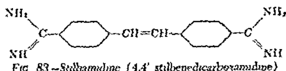


Fig 83 — *Stilbamidine (4,4' stilbenedicarboxamidine)*

but without causing trigeminal neuropathy or disturbance of kidney function, hence it could be administered in much larger doses The 2-hydroxystilbamidine compound is more stable in solution and less toxic than stilbamidine and it inhibits the growth of leishmaniae, *Blastomyces dermatitidis* and *Histoplasma capsulatum* equally well The successful treatment of several cases of North American blastomycosis with 2-hydroxystilbamidine was reported subsequently by Snapper and his associates[394,396] Treatment in adults consisted of intravenous administration of 110 mg 2-hydroxystilbamidine di-isethionate* contained in 20 ml 5 per cent glucose in normal saline administered intravenously over a 20 minute period every other day until daily doses could be tolerated One patient received a total of 19 6 grams 2-hydroxystilbamidine di-isethionate, and another received 8 1 grams during fifty-two days of treatment A child with North American blastomycosis was treated by daily infusions of 2-hydroxystilbamidine di-isethionate. Administration was at first intravenous (eight weeks) then intramuscular (seven days), using a 75 mg dose contained in 2 cc 5 per cent procaine-glucose solution.[394] The drug was well tolerated by intramuscular route and no evidence of toxic neuropathy was observed In the case of a patient who had received large amounts of 2-hydroxystilbamidine, skin biopsy with fluorescent microscopy revealed the drug to be present in the epidermis, sweat glands, cutaneous nerves, and in the inflammatory exudate In patients in whom clinical cures were obtained consistently, it was observed that the strains of *B dermatitidis* isolated in each instance had a different degree of sensitivity to 2-hydroxystilbamidine (e g, 1, 15, 30, and 45 μg/ml) and, in two cases, seemed to be relatively resistant The discrepancy between favorable

* Contains 54% 2-hydroxystilbamidine (furnished by William S Merrell Co)

duced the inhibitory effect even further. In vitro experiments by Gonzales and associates[153,154] demonstrated that copper sulfamerazine and copper sulfathiazole stimulated rather than inhibited the growth of *C. neoformans.*

Blood levels of sulfapyridine, sulfanilamide and sulfadiazine achieved in patients with cryptococcal meningitis were 9–13 mg. per cent, 9–12 mg. per cent, and 8–13 mg. per cent, respectively.[126,151,339,439] Spinal fluid levels of sulfapyridine and sulfadiazine have been found to be 1.7–5.0 mg. per cent and 4.5–7.1 mg. per cent, respectively.[68,73,126]

It is obvious, therefore, that concentrations of sulfonamide obtained in the blood and spinal fluid of patients are far below the minimal concentration necessary for inhibition of the organism. In the case of sulfadiazine, for example, the spinal fluid level achieved is only 1/400th the required inhibitory concentration. However, depending upon the pharmacologic properties and biologic nature of the drug employed, there may not be a direct relationship between in virto fungistatic activity and in vivo curative effect (see Diamidines, page 132). The potential leukopenic effect of sulfonamide is considerable, and, for this reason, therapy has been discontinued in some cases of cryptococcal meningitis.[207] In view of the patient's poor immunologic response, it is possible that leukopenia-inducing drugs such as the sulfonamides may do more harm than good.

In 1942, Marshall and Teed[279] reported the case of a 9 year old white girl with bilateral cryptococcal mastoiditis and localized cryptococcal meningitis, treated by sulfadiazine and bilateral mastoidectomy. Blood and spinal fluid levels of sulfadiazine were 6.0 mg. per cent and 5.0 mg. per cent respectively. Eleven years later the patient was still alive, asymptomatic, and reported free of infection, although spinal puncture had not been performed since the case was first reported.[280] The above authors were "impressed by the apparent curative effect of sulfadiazine." Following their original report, sulfonamide therapy of patients with cryptococcal meningitis was reported by other investigators (Table VI), and the various sulfonamide drugs have received a thorough clinical trial but with uniformly poor results.

Diamidines: In view of the serious prognosis of cryptococcal meningitis and its resistance to therapy, a report of the successful treatment of a case of generalized cryptococcosis with 2-hydroxystilbamidine is of considerable interest.[461] *Clinical trial of diamidine derivatives in North American* blastomycosis and other mycoses was first stimulated by Elson's report[104] of the inhibitory effect of propamidine* on *Blastomyces dermatitidis, Sporotrichum schenckii* and other fungi. Schoenbach and associates[366,367] successfully treated 4 patients with cutaneous and systemic North American blastomycosis, using propamidine and stilbamidine†, and many others have reported equal success.[16,74,85,311,315,406] The effectiveness of stilbamidine in suppressing pulmonary infection in mice was reported by Heilman.[176] Propamidine and stilbamidine (Fig. 83) are members of a series of aromatic diamidines introduced originally for the treatment of kala-azar[1] and more recently multiple myeloma.[193]

Stilbamidine is a toxic drug with both immediate and delayed manifesta-

* (4,4' diamidino 1,3-diphenoxypropane)

† (4,4' stilbenedicarboxamidine)

seventeen consecutive days for a total of 4 05 Gm. Marked subjective improvement occurred, and by the fourteenth day of treatment the temperature had returned to normal from a level of 103°F. The patient was discharged after thirty days' hospitalization, at which time liver, spleen, and lymph nodes were no longer tender or palpable. This patient apparently did not have meningeal infection, and, for that matter, it is uncertain that all her initial clinical manifestations were due to cryptococcosis. Since the diamidine drugs have been used to advantage in multiple myeloma, the good results obtained in this case are not necessarily indicative of the control of the infectious process The authors have informed us that twenty-one months later there was no evidence of recurrence Before this encouraging experience can be evaluated properly, further follow-up of this case and treatment of other patients with this drug will be necessary.

Ethyl vanillate:* This by-product in the manufacture of sulphite pulp is an ethyl ester of vanillic acid with the formula ethyl 4-hydroxy-3-methoxy benzoate ($C_{10}H_{12}O_4$).[70,316] It inhibits *Histoplasma capsulatum* at concentrations of 200–400 μg/ml,[59,70,107] but has the disadvantage of causing a burning sensation of the mucous membranes. Lane[247] administered the drug orally to a 12 year old girl with cryptococcal meningitis, at a rate of 3 5 grams every four hours After nine days, intractable vomiting set in and the child refused further medication. Shortly thereafter she became asymptomatic and was discharged from the hospital, but was readmitted 6 months later and succumbed to the disease before the drug could be administered a second time Christie and associates[70] have emphasized that the margin between effective therapeutic dose and toxic dose of ethyl vanillate is too narrow for it to be considered a desirable therapeutic agent Blood levels above 50 mg per cent (500 μg/ml) increased the toxic manifestations, which consisted of drowsiness, apathy, skin eruption, respiratory alkalosis, and possibly liver necrosis and degeneration of proximal convoluted renal tubules Daily administration of ethyl vanillate by syringe gavage was ineffective in delaying mortality in *Histoplasma*-infected white Swiss mice[493]

Esters of parahydroxybenzoic acid: McVay and Sprunt† demonstrated by in vitro and in vivo experiments that esters of parahydroxybenzoic acid exerted a definite inhibitory effect on five *Candida* species Methyl and propyl esters of paraben, given to thirteen patients in a daily dose of 800 mg for four days, were tolerated without toxicity They were also nontoxic when instilled into the vagina and rectum More detailed studies by Siegel[illegible] revealed methyl parahydroxybenzoate (methyl paraben) to be inhibitory in vitro for *C neoformans* at 275 μg/ml and propylparahydroxybenzoate at 50 μg/ml By combining methylparaben and propylparaben, an additive but not synergistic inhibitory effect was obtained Wolf[471] reported that p-hydroxy methyl benzoate inhibited *C neoformans* in vitro in concentration of 0 05 per cent (500 μg/ml)

Thiosemicarbazones. Compounds of the thiosemicarbazone group have been tested for inhibitory action on *C neoformans*[203] Complete inhibition was noted as follows

* E R Squibb & Sons, New Brunswick, N J

† Proc Soc Exper Biol & Med 78 759–761, 1951

clinical results and in vitro resistance may be explained in part by the drug's pharmacologic property of concentrating in infected tissues and inflammatory cells. Thus it is possible that infection with fungus species such as *C. neoformans*, with even greater resistance in vitro, may be controlled by 2-hydroxystilbamidine.

Diamidine derivatives have been employed experimentally and clinically in the treatment of cryptococcosis. Fisher[124] found that two strains of *C. neoformans* were destroyed by propamidine in concentration of 0 062 per cent (620 μg/ml.) and 0 25 per cent (2500 μg/ml.) and inhibited by 0.008 per cent (80 μg/ml.). In vitro studies by Solotorovsky and associates[197] showed propamidine and hydroxystilbamidine to be the most active diamidines against *C. neoformans* (see Table VIII, below). One of our recent isolates of *C. neoformans* from a fatal human case was inhibited by 67 5 μg/ml 2-hydroxystilbamidine* and by 125 μg/ml. 2-aminostilbamidine* Fisher[124] found that propamidine did not protect mice against *C. neoformans* infection, and Miller and his associates[249] were equally unsuccessful with stilbamidine in mice infected by the cerebral route. An unsuccessful clinical trial of stilbamidine in three cases of cryptococcal meningitis was reported by Miller and associates[287] Intravenous administration of the drug failed to affect the clinical course of the disease The authors felt that this was consistent with the relative resistance in vitro of *Cryptococcus neoformans* to diamidine drugs when compared with the sensitivity of *Blastomyces dermatitidis*[104,124,397] (Table VIII)

TABLE VIII —IN VITRO ACTIVITY OF DIAMIDINE DRUGS
(After Solotorovsky et al [197])

	C neoformans	*B dermatitidis*
Stilbamidine	50 μg/ml	<2 5 μg/ml
Stilbamidine +1% albumin	100	5
Hydroxystilbamidine	25	<2 5
Hydroxystilbamidine +1% albumin	50	<2 5
Pentamidine*	100	25
Phenamidine†	>100	50
Propamidine‡	10	2 5

* 4,4' (pentamethylenedioxy) dibenzamidine
† p,p' oxydibenzamidine
‡ 4,4' diamidino 1,3-diphenoxypropane

Whitehill and Rawson,[461] however, reported the successful treatment with 2-hydroxystilbamidine of a 16 year old Negro girl believed to have generalized cryptococcosis but no intracranial involvement The patient had generalized adenopathy and splenomegaly which were considered due to Hodgkin's disease until biopsy and culture of a supraclavicular lymph node led to a diagnosis of cryptococcosis. Treatment with 2-hydroxystilbamidine was instituted, 75 mg contained in 200 ml 5 per cent glucose was administered by slow intravenous drip the first day, 150 mg the second day, and 225 mg daily thereafter for

* Furnished by William S Merrell Co , Cincinnati, Ohio

seventeen consecutive days for a total of 4 05 Gm. Marked subjective improvement occurred, and by the fourteenth day of treatment the temperature had returned to normal from a level of 103°F. The patient was discharged after thirty days' hospitalization, at which time liver, spleen, and lymph nodes were no longer tender or palpable This patient apparently did not have meningeal infection, and, for that matter, it is uncertain that all her initial clinical manifestations were due to cryptococcosis Since the diamidine drugs have been used to advantage in multiple myeloma, the good results obtained in this case are not necessarily indicative of the control of the infectious process. The authors have informed us that twenty-one months later there was no evidence of recurrence. Before this encouraging experience can be evaluated properly, further follow-up of this case and treatment of other patients with this drug will be necessary.

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Tibione (4-acetylaminobenzal thiosemicarbazone)	250 µg/ml
Myvisone (p-formylacetanilide thiosemicarbazone)	250 µg/ml.
Vanithiazone	100 µg/ml
Bromothiazone	50 µg/ml

Thiocarbamate derivatives: Urethan (ethyl carbamate), employed in treatment of lymphoblastoma, is one of a group of compounds used to control fungus plant disease.[341] Kligman and Weidman[220] tested six carbamates and noted that, although they exerted a marked fungistatic effect in vitro on *C. neoformans* (1:50,000 or 20 µg/ml.), even in the presence of blood, and were of low toxicity, carbamates were unsuccessful in protecting mice infected with cryptococci

Thiourea derivatives: Both dithiocarbamyl hydrazine and thiocarbamyl hydrazine inhibited *C. neoformans* at 40 mg. per cent (400 µg/ml), in the presence and absence of human serum, however, dithiocarbamyl hydrazine failed to influence the fatal course of cryptococcal-infected mice.[418]

Compound P-594: Drugs of the type $ArCH(OR)CBr(NO_2)R'$ have been noted to possess strong antifungal activity[190] A compound designated P-594 (2-bromo-1-methoxy-2-nitro-1-phenylpropane) inhibited *Blastomyces dermatitidis* in 1 µg/ml, *Candida albicans* in 10–25 µg/ml, and *Saccharomyces cerevisiae* in 10–25 µg/ml No results were reported for *C neoformans.* The compound was well tolerated in 1 per cent concentration in vanishing cream when applied to the skin

Fatty acid derivatives: Pelargonic, capric and undecylenic acids were the most effective of the 13 fatty acids studied in vitro by Kligman and Weidman,[220] but whole blood added to the medium reduced the fungistatic effect. None of the following compounds afforded protection to mice inoculated with cryptococci by the intraperitoneal route. (1) sodium pelargonate 500 mg./Kg, (2) sodium caprate 150 mg /Kg. or (3) sodium undecylenate 400 mg /Kg

β-diethylaminoethyl fencholate: This organic compound originally described by Tilford and co-workers[425] and designated MRD-112* was found by Ludwig and associates[264] to be an effective antifungal agent When compared with gliotoxin, a highly active but toxic antifungal agent, as well as with diamthazole, nitrofurfuryl methyl ether, and copper-3-phenyl salicylate, it possessed comparable activity in inhibiting *C neoformans* as follows

Gliotoxin	100 µg/ml
Diamthazole	>500 µg/ml
Nitrofurfuryl methyl ether	500 µg/ml
Copper-3-phenyl salicylate	>500 µg/ml
MRD-112	500 µg/ml

LD_{50} in mice for MRD-112 was found to be 72 mg./Kg, while that for diathazole was 98 mg /Kg

Naphthoquinones and related compounds: The fungistatic properties of 2,3-dichloro-1,4-naphthoquinone were first reported by Ter Horst,[421] and the drug was applied for the treatment of superficial mycoses[218] Its fungistatic activity in vitro, as well as that of seventeen other naphthoquinones, including Mena-

* β-diethylaminoethyl 1-methyl-3-isopropyl cyclopentanecarboxylate hydrochloride, Wm S Merrell Co, Cincinnati, Ohio

dione, was considerably reduced by whole blood Five quinone derivatives failed to protect mice infected intraperitoneally with *C neoformans*.[220]

Quinacrine (Atabrine) was r

centrations of 25 to 50 mg per ce

mg per cent (30–250 μg/ml.).[42]

μg/ml. may cause toxic manifestations in humans and these levels are rarely found in patients treated conservatively with this drug,[244] it is of little therapeutic value in cryptococcal infections [42]

Antihistaminic compounds: The inhibitory effect of Pyribenzamine hydrochloride (Ciba) Antistine hydrochloride (Ciba) and diphenylpyraline (Nopco) was reported for *C neoformans* at levels of 1000, 250 and 500 μg/ml, respectively, which was reduced by the addition of horse serum [33] Assuming that all tissues

value in cryptococcal infection

Isonicotinic acid hydrazide: Instead of an inhibitory effect, isonicotinic acid hydrazide caused enhancement of growth of *C neoformans* and other pathogenic fungi at concentration of 0 8 mg /ml. (800 μg/ml), although some inhibitory effect was noted at 1.0 mg /ml [31] This observation would be of clinical importance for patients with co-existing tuberculosis and fungus disease who might be receiving this drug

Miscellaneous compounds: The most comprehensive laboratory evaluation of chemotherapeutic drugs for cryptococcosis was undertaken by Kligman and Weidman,[220] who made tests in vitro of several hundred miscellaneous compounds, none of which was effective in dilution 1 500 (2000 μg/ml) A partial list follows

antimony potassium tartrate
dichlorophenarsine
diethylstilbestrol
hyamine 10x
hyamine 1622
N-butylmaleimide
Neoarsphenamine
nicotinamide
nitrofurazone (Furacin®)
p-aminobenzoic acid
p-aminohippuric acid
p-nitrobenzoic acid
phenazine di-N-oxide
phenothiazine
propamidine
pyrimidine
quinacrine (Atabrine®)
quinine
resorcinol
sodium p,p'diaminodiphenylsulfone-N,N'-didextrose sulfonate (Promin®)
stibophen (Fuadin®)
2-(dimethylaminomethyl)-1-naphthol hydrochloride
2-metanilamido-5-chloropyrimidine
3-phenyl hydantoin
4,2'-diaminophenyl-5' thiazolylsulfone (Promizole®)
4,4'-dihydroxy-3,3'di (diethylaminomethyl)-5,5'-diallyl-diphenyl dihydrochloride
5 amino-7-hydroxy-1-triazole
6-cyano-2-methoxy-9-isopropylaminopropyl-aminoacridine
6-methoxy-8-isopropyl-amino-pentylamino-quinoline
6-methoxy-8-morpholino-propylamino quinoline
6-phenyl-5,6-dihydrouracil

Daraprim® (see Addendum, page 149)
Chlorquinaldol (see Addendum, page 150)

c. ***Antibiotic therapy:*** Antibiotics have been employed successfully in the treatment of bacterial and viral diseases of man, but they have not proved useful for the treatment of cryptococcosis (Table VI). Although many antifungal antibiotics produced by bacteria, actinomycetes and fungi have been found to be effective in vitro against *C. neoformans* (Tables IX, X, XI), their high toxicity and ineffectiveness in vivo has rendered them of little clinical value.

TABLE IX —ANTIFUNGAL SUBSTANCES DERIVED FROM BACTERIA

Antifungal substance	Derived from	In vitro inhibition of *C neoformans*	Toxicity for mouse
Prodigiosin[182,281,293]	*Chromobacterium prodigiosum*	—	Nontoxic in amounts therapeutically effective for *C. immitis* infection
Violacein[242,243]	*Chromobacterium violaceum*	—	1–2 mg tolerated (intraper.)
Pyocyanine[182,408]	*Pseudomonas aeruginosa*	—	MLD. 2 mg (intraper.)
Hemi-pyocyanine*[268,408]	*Pseudomonas aeroginosa*	—	MLD 5 mg (intraven)
Circulin[272,292,423]	*Bacillus circulans*	8–15 μg/ml	LD_{50} 23 mg /Kg (intraven)
Polymyxin[59,127,401]	*Bacillus polymyxa*	3 μg/ml	LD_{50} 300 mg /Kg (subcut)
Bacillomycin †[220,230,379]	*Bacillus subtilis*	20–50 μg/ml	LD_{50} 75 mg /Kg (intraper)
Eumycin †[204]	*Bacillus subtilis*	*None*	*5 mg tolerated (intraven)*
Fungistatin[168] (Antibiotic XG)	*Bacillus subtilis*	14–21 μg/ml. (65–100 units/mg)	LD_{50}· 90 mg /Kg (intraven)
Mycosubtilin[449]	*Bacillus subtilis*	5 μg/ml	MLD 1 mg (0 25 mg *tolerated)*
Fluvomycin[61]	*Bacillus subtilis*	13–20 μg/ml (50–75 units/mg)	LD_{50} 1 3 Gm /Kg (intraven)

* Alpha-hydroxy phenazine

† Both considered similar by Sharon et al [379] and to seven other antifungal extracts produced by *Bacillus subtilis*

Patients with cryptococcal meningitis have been treated with the following antibiotics without materially altering the course of the illness· Actidione, Aureomycin, Chloromycetin, circulin, penicillin, polymyxin, prodigiosin, protoanemonin, streptomycin, Terramycin and tyrocidin

Kligman and Weidman's[220] extensive studies in vitro revealed that the effective antibiotics of antifungal nature were Actidione, protoanemonin, bacillomycin, pleurotin and allicin Nevertheless, none of these afforded protection to mice infected with cryptococci *Antibiotics which were found to be ineffective in vitro* were crude penicillin, penicillin G, penicillin K, penicillic acid, Chloromycetin, Aureomycin, streptomycin, bacitracin, tyrothricin, tyrocidin, gramicidin, subtilin, gladiolic acid, crepin, kojic acid and aspergillic acid[220] Hobby and *associates*,[187] who had reported anti-cryptococcal activity of culture filtrates of *Penicillium notatum*, probably were working with unpurified fractions.

Antifungal substances derived from bacteria: Antifungal antibiotics produced by bacteria are: prodigiosin, violacein, pyocyanine, hemi-pyocyanine, circulin, polymyxin, bacillomycin, eumycin, fungistatin (Antibiotic XG), mycosubtilin, fluvomycin (Table IX). Three of these compounds have been administered unsuccessfully to patients with cryptococcal meningitis, i e, prodigiosin,[21,347] circulin, which was nephrotoxic,[421] and polymyxin [234,329]

More than a score of antifungal substances have been extracted from *Bacillus subtilis* since the first report of Lewis, Hopper and Schultz,[240] who noted antifungal activity of culture filtrates of this organism. Landy and associates[230,231] described bacillomycin, derived from *Bacillus subtilis,* which inhibited *C. neoformans* at a concentration of 0 05 mg /ml (50 μg/ml.). Nine antifungal agents subsequently extracted from *Bacillus subtilis* by various workers were shown by Sharon and associates[379] to be similar, i e, they were all extracellular, acidic, nondialyzable and heat-stable polypeptides, not affected by pepsin or trypsin,

TABLE X—ANTIFUNGAL SUBSTANCES DERIVED FROM FUNGI

Antifungal substance	Derived from	In vitro inhibition of *C neoformans*	Toxicity for mouse
Clavicin[240,444,468]	*Aspergillus clavatus*	100 μg/ml	LD$_{50}$ 0 5 mg /Kg (intraven)
Citrinin[161,220,335]	*Penicillium citrinum*	1,000 μg/ml.	LD$_{50}$ 35 mg /Kg (intraper)
Gliotoxin[261,340,455]	*Trichoderma*	100 μg/ml (05 μg/ml)*	LD$_0$ 45–65 mg /Kg (intraper)
Pleurotin[220,349]	*Pleurotus griseus*	10 μg/ml	24 mg /Kg tolerated (intraven.)
Trichothecin[144]	*Trichothecium roseum*	—	—
Viridin[40,41,290]	*Trichoderma viride*	200–500μg/ml	—
Ustilagic acid[159]	*Ustilago zeae*	10–15μg/ml	1500 mg /Kg tolerated (oral)

* One gram contains 20,000,000 units (Reilly et al [349])

and *all* possessed similar properties with respect to solubility All nine substances were therefore classified as "bacillomycin" Three additional antibiotics not satisfying these criteria were fungistatin (Antibiotic XG),[188] mycosubtilin,[449] and rhizoctonia factor [285] Another dialyzable antibiotic derivative of *B. subtilis,* active against *C neoformans* and named fluvomycin, was reported by Carvajal.[61] *Although the* compound was of a low order of toxicity in mice (Table IX, page 136), Carvajal was unable to demonstrate protective action

To our knowledge, the *Bacillus subtilis* derivatives have not been administered to patients with cryptococcal meningitis The derivatives should be withheld until they are purified to a higher degree and rendered less toxic.

Antifungal substances derived from fungi. Antibiotics exhibiting antifungal activity and derived from fungi are clavicin, citrinin, gliotoxin, pleurotin, trichothecin, viridin, and ustilagic acid (Table X) Pleurotin and viridin were noted to be inhibitory in vitro to *C neoformans* in concentrations of 10 μg/ml and 200 μg/ml, respectively, but the former drug was ineffective in treating mice in-

[illegible] neal *route* [220] *The most promising of these*

which is a partially acylated derivative of

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fected with [illegible]
drugs appe[illegible]
di-D-glucosyl-dihydroxyhexadecanoic acid obtained by Haskins and Thorn[169]

from *Ustilago zeae*. It inhibits *C. neoformans* at 10–15 μg/ml. and is relatively nontoxic to mice and rats on oral administration. Protection tests were not reported.

Antifungal substances derived from actinomycetes: Alexopoulos[3] pointed out that as many as 56 per cent of all cultures of actinomycetes possessed some

TABLE XI.—ANTIFUNGAL SUBSTANCES DERIVED FROM ACTINOMYCETES

Antifungal substance	Derived from	In vitro inhibition of *C neoformans*	Toxicity for mouse
Actinomycin[340,447]	*Streptomyces antibioticus*	0 6 μg/ml	extremely toxic, 10 μg kills mouse in 24–48 hours
C-381[446]	*Streptomyces* (WC 3569)	1 4 μg/ml	—
C-135[446]	*Streptomyces* (WC 3570)	<2 5 μg/ml	—
Musarin[8,415]	*Streptomyces* sp	—	—
Streptothricin[133,220,240,351,448]	*Streptomyces lavendulae*	250–500 μg/ml	LD_{50}: >2630 units (intraven)
Streptomycin[351,363,372]	*Streptomyces griseus*	>4,000 μg/ml	LD_{50}: 250 mg /Kg (intraven) LD_{50}: 1500 mg /Kg (subcut)
Actidione[446,459,460]	*Streptomyces griseus*	0 21 μg/ml	LD_{50}: 150 mg /Kg (intraven)
Candicidin[218,334]	*Streptomyces griseus* (No 3570)	0 5–1 0 μg/ml	LD_{50}. 47–79 mg /Kg (intraper), 1% sol subcut causes necrosis
Rimocidin[59,68,376]	*Streptomyces rimosus*	5–10 μg/ml	LD_{50} 20–30 mg /Kg (intraven)
Thiolutin[59,375]	*Streptomyces albus*	5–25 μg/ml	LD_{50}. 25 mg /Kg (subcut)
Fradicin[184]	*Streptomyces fradiae*	2–4 μg/ml	LD_{50} 4 mg /Kg (intraper)
Ascosin[183]	*Streptomyces canescus*	1 2 units/ml	LD_{50} 8 6 mg /Kg (intraper)
Candidin[417]	*Streptomyces viridoflavus*	—	LD_{50} 7–36 mg /Kg (intraper)
Fungicidin[173,330] (nystatin)	*Streptomyces noursei*	1 56 μg/ml	LD_{50} 20–26 mg /Kg (intraper)
Fungichromin[498]	*Streptomyces cellulosae*	—	Toxic dose 16 4 mg /Kg (intraper)
Mycoticin[478]	*Streptomyces ruber*	6-8 μg/ml	LD^{50} 10–20 mg /Kg (intraper)

antifungal substances, one-third of these being strong inhibitors of fungi This is illustrated by the large number of antifungal substances derived from the actinomycetes listed in Table XI, the most recent being Actidione, nystatin, candicidin, ascosin, fradicin, rimocidin, thiolutin, and candidin Of these, Actidione has received the most extensive clinical trial in cryptococcal meningitis, but with poor clinical results

Nystatin (fungicidin, Mycostatin),* derived from *Streptomyces noursei*,[173] is a promising antibiotic which is strongly fungicidal but lacks antibacterial activity. It is inhibitory to *C neoformans* in concentration of 1 56 μg/ml[173] and is well tolerated intraperitoneally by mice in dosage of 24 mg /Kg, by dogs in dosage of 1 Gm /Kg, and by rats in dosage of 300 mg./Kg, by gastric lavage. The survival time of mice infected intravenously with cryptococci was extended beyond that of the control group by parenteral nystatin[173]

Nystatin was administered orally to two patients at the Mt Sinai Hospital, New York in 1955 without beneficial result. One patient with lymphosarcoma, generalized moniliasis and cryptococcosis received orally 1,500,000 units of nystatin every day for four days, but subsequently died A second patient with Hodgkin's disease and generalized moniliasis and cryptococcosis received 3,000,000 units of nystatin orally every day for seven days and also died An intravenous preparation containing 200,000 units per 2 cc vial, now available for administration with 5 per cent glucose, may prove more efficacious The potential parenteral toxicity of nystatin, however, must be carefully considered For example, after the fourth 0 5 mg dose of nystatin had been injected subcutaneously into *Histoplasma*-infected mice, suppurative lesions appeared at the site of injection and 2 to 3 weeks were required for healing after discontinuance of therapy[84] The complete reversal of the fungicidal activity of fungicidin, an antibiotic identical with nystatin, by M/100 cysteine hydrochloride,[336] indicates that antibiotics of the fungicidin type may be rendered ineffective in vivo Fatty acids and cysteine were found to reduce the activity of nystatin[480] (see Addendum, page 148, for additional data on nystatin)

Candicidin is an antibiotic produced by *Streptomyces griseus* No 3570 which inhibits *C neoformans* in a concentration of 0 5 μg/ml (Lechevalier and associates[234]) The median lethal dose (LD_{50}) for the white mouse was reported to be 47–79 mg /Kg[216 234] After *C neoformans* had been injected intravenously, candicidin was not effective in prolonging survival of the mice or in altering mortality rate[216]

Ascosin is an antifungal antibiotic which inhibits *C neoformans* in 1 2 units/ml and is derived from *Streptomyces canescus*[183] When administered to mice infected intraperitoneally with *Histoplasma capsulatum*, doses approaching toxic levels were required to produce a therapeutic effect, leading to the conclusion that in its present form, ascosin was too toxic for use in humans[111]

Actidione† (cycloheximide) This promising fungicidal antibiotic has now received an extensive trial in the treatment of cryptococcal meningitis Waksman, Schatz and Reilly[446] first reported Actidione in culture filtrates of *Streptomyces griseus*, the well known streptomycin-producing organism The following year Whiffen, Bohonos and Emerson[466] described the marked fungicidal properties of Actidione for *C neoformans*, for which it was inhibitory in a dilution of 1 1,300,000 Subsequent studies confirmed this inhibitory concentration at 0 24 μg/ml[459] The acute toxicity of Actidione determined in experimental animals varied from 2 5 mg /Kg for rats to 150 mg /Kg for mice (see Table XII, page 140)

* E R Squibb & Sons, New Brunswick, N J

† Upjohn Co , Kalamazoo, Michigan

TABLE XII.—LD_{50} VALUES OF ACTIDIONE IN ANIMAL SPECIES
(*After Whiffen*[459])

Species	Mode of Administration	LD_{50} Dose
Mouse	intravenously	150 mg./Kg.
Guinea pig	subcutaneously	60 mg./Kg.
Rabbit	intravenously	17 mg./Kg.
Cat	intraperitoneally	4 mg./Kg.
Rat	subcutaneously	2.7 mg./Kg.
Rat	intravenously	2.5 mg./Kg.

Chemical studies of the compound have revealed it to be a di-ketone containing one hydroxyl and only one ketonic group (Fig. 84) with the empirical formula $C_{15}H_{23}NO_4$.[132,222] Actidione is rapidly inactivated by dilute alkali at room temperature.[233] Several assay methods for Actidione have been described. A filter paper disc-agar plate diffusion technic, first described by Loo and associates[261] for streptomycin assay, was adapted by Whiffen[459] for testing the activity

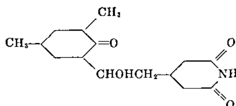


FIG. 84.—Actidione (cycloheximide), after Kornfeld and Jones[222]

of Actidione against *Saccharomyces pastorianus* ATCC* 2366. Another satisfactory technic is the standard tube dilution method employing liquid culture media.

In attempting to standardize antifungal serum assay methods, Tarbet and Sternberg[420] described a microbiologic technic for the estimation of antifungal substances in serum, utilizing *Candida tropicalis* (Castellani) Berkhout† as the test organism. The method involves counting of yeast cells in hemocytometer after exposure to a liquid buffered asparagine dextrose medium containing definite quantities of fungicides as compared with test serum in the same medium. By comparing the number of cells in both series of tubes, the antifungal activity of the test serum may be calculated in terms of micrograms of drug per unit volume of serum. Assays of guinea pig sera drawn 3 hours after intraperitoneal injection of 16 mg./Kg. of fungicides failed to demonstrate the presence of Actidione, but showed the presence of other antimycotic agents in appreciable amounts (see Table XIII). The failure of Actidione to reach the blood stream, however, does not necessarily mean that it is an ineffective drug. Stilbamidine isethionate, also absent from guinea pig serum by the same testing technic, has proved to be effective in the treatment of animal and human North American blastomycosis (see Diamidines, page 130).

* American Type Culture Collection, Washington, D. C.
† Maintained at the Department of Microbiology, Rutgers University, New Brunswick, N. J.

TABLE XIII.—BLOOD SERUM LEVELS OF FUNGICIDES IN THE GUINEA PIG 3 HOURS AFTER INTRAPERITONEAL INJECTION OF 16 MG/KG DRUG

(After Tarbet and Sternberg[410])

Compound	Serum level μg/ml	LD_{50} in Mouse
Actidione	0	150 mg./Kg. (intraven.)[169]
Prodigiosin	0	Nontoxic in amounts therapeutically effective for C. immitis infection[229, 467]
Stilbamidine isethionate	0	100 mg./Kg. (orally)*[370]
Rimocidin	0.75	20-30 mg./Kg. (intraven.)[88, 374]
Ascosin	1.7	8.6 mg./Kg. (intraper.)[185]
Fungicidin (Nystatin)	22.7	20-26 mg./Kg. (intraper.)[178]
Candicidin	28.0	47-79 mg./Kg. (intraper.)†[224]

* Caused fatty metamorphosis of the liver and degeneration of the renal convoluted tubules.[370]

† Toxic at three-fold minimal therapeutic dose for C. albicans in mice.[224]

Sources

...heximide)

...odiniosum)

... Brooklyn,

... Corp., Terre

... griseus No. 3570

... source: E. R. Squibb and Sons, N. Y.

...

... and Weidman[336] reveal that Actidione affords no pro... ...tococci when the dosage was 25 mg./kg. ... for one day. In view of the high degree of susceptibility of the ... this antibiotic in vitro and the fact that Actidione is not inactivated by whole blood, no explanation was found by the authors for this therapeutic failure. Fisher[131] noted that Actidione inhibited strains of C. neoformans in concentrations of 1 to 8 μg/ml., yet the antibiotic was ineffective in the treatment of mouse infection. Carton[58] reported Actidione to inhibit 8 human isolates of C. neoformans with 0.04 to 6.0 μg/ml. Later experiments in vivo revealed no significant differences in average days of survival between infected control and treated animals,[59] also that Actidione was fungistatic rather than fungicidal. Our own strains of C. neoformans varied in susceptibility to Actidione from 0.1 to 0.2 μg/ml.

The first case to be treated with Actidione was that of a 26 year old white woman with cryptococcal meningitis, in coma, who received a total of 1400 mg. of Actidione without avail.[391,141] After preliminary toxicity study in a rhesus monkey, Actidione was given to the patient intramuscularly, intravenously and intrathecally. When 150 mg. of the drug had been administered by various routes in five days, spinal fluid cultures became negative. Although ten subsequent spinal fluid cultures remained negative, cryptococci reappeared

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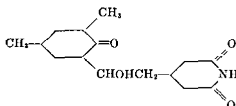


FIG. 84.—Actidione (cycloheximide), after Kornfeld and Jones[222]

of Actidione against *Saccharomyces pastorianus* ATCC* 2366. Another satisfactory technic is the standard tube dilution method employing liquid culture media.

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* American Type Culture Collection, Washington, D. C.

† Maintained at the Department of Microbiology, Rutgers University, New Brunswick, N. J.

intrathecally in doses of 4 5 to 10 mg daily, and intraventricularly in doses of 20 to 30 mg. daily. In one case, the daily intrathecal administration of 20 mg doses of Actidione for fourteen days was accompanied by signs of marked cerebral irritation: lethargy, mental depression, slurred speech and ataxia, which did not resolve until three weeks after discontinuance of the drug [479]

The poor clinical results experienced with Actidione may be due to the relatively small parenteral doses administered The highest intravenous dose given to a patient in a 24-hour period was 180 mg, which corresponds to 2 5 µg/Kg for a 70 Kg individual [101] This is approximately one-half the amount of Actidione lethal for a rat, one-thirteenth the amount lethal for a rabbit, and one-one-hundred-twentieth the amount lethal for a white mouse (see Table XII). There are indications that human beings can tolerate much larger doses intramuscularly, for example, in Carton's Case 4, 2631 mg. of Actidione were given intramuscularly in one day without evidence of toxicity. Inhibitory concentrations of Actidione to human isolates of *C neoformans* vary from 0 04 to 8 µg/ml [58,59,171] If Actidione were completely and equally absorbed by all tissues, it would require approximately a 700 milligram dose to achieve a tissue concentration of 10 µg/ml. in a 70 Kg individual, assuming that there was no conjugation by the liver or excretion by the kidneys, lungs or skin

In one fatal case of cryptococcal meningitis reported from England,[33] the patient received daily 60 mg Actidione intravenously and 20 mg intrathecally The patient's own Seitz-filtered, spinal fluid failed to inhibit the strain of *C neoformans* isolated from him, and a second strain, indicating the failure of the medication to reach fungicidal level If Actidione exhibited the pharmacologic property similar to that of penicillin in not reaching appreciable levels in the spinal fluid and central nervous system after intramuscular or intravenous injection, its usefulness for the treatment of cryptococcal meningitis would be seriously curtailed This point could be clarified by experiments with larger animals, such as dogs or sheep, for detection of spinal fluid levels following large intravenous and intramuscular injections of Actidione

Fungichromin (see Addendum, page 147)

Mycoticin (see Addendum, page 148)

Antifungal substances derived from higher plants: Seegal and Holden[312] extracted a lactone known as protoanemonin from the buttercup, *Anemone pulsatilla*, which is a member of the plant family Ranunculaceae The compound protoanemonin is vesicant in nature, causes sneezing and lacrimation, and has the formula 2,4-pentadiene-1,4-olide [261] Mice tolerated eight successive intraperitoneal doses of anemonin of 50 mg/Kg without any loss in weight [261] It was noted to inhibit *C neoformans* [193,312] The fungistatic effect of protoanemonin was reported to be 1 10,000 (100 µg/ml) but protective action for mice infected intraperitoneally with cryptococci could not be demonstrated [270] Protoanemonin appeared to cure a cryptococcal bone lesion[54,76] and was subsequently administered by Carton[56] to four patients with central nervous system involvement One patient received 4 to 10 mg in 10 ml saline intrathecally twice a week for two months for a total dose of 100 mg Although temporary improvement occurred in two patients, the drug was definitely toxic, as it caused signs of root irritation and neurogenic bladder. Despite the activity in

in later specimens. The patient died ten weeks after admission to the hospital (five weeks after Actidione therapy had been started) Autopsy revealed meningoencephalitis of an unusually granulomatous character (Figs 53A, 60 and Plate IE) The intense host response to the fungus may have been related to the destruction of cryptococci by Actidione; however, viable cryptococci were cultured from several sites Blood levels of Actidione were not demonstrable in this patient, even though specimens were drawn from the opposite arm while a continuous intravenous drip was running

Haspel and associates[107] reported a case of systemic and cerebral cryptococcosis with positive blood and spinal fluid cultures, who was treated intramuscularly with 40 mg. Actidione daily Actidione in concentration of 10 μg/ml. inhibited the organism Although there was initial clinical improvement the condition of the patient deteriorated rapidly and death occurred on the twentieth day of therapy (sixty days after hospital admission). Autopsy revealed cryptococcal involvement of the brain, spinal cord, pericardium, kidneys, adrenals and lungs

Levy[238] treated a 58 year old woman with a cryptococcal lesion of the lung. The patient previously underwent operation for removal of a cryptococcal granuloma of the cerebellum and subsequently developed cryptococcal meningitis She was treated with 30 mg Actidione intramuscularly, 10 mg daily intrathecally and 20 to 30 mg daily intraventricularly Spinal fluid cultures were negative twenty-four hours after therapy had been initiated Although the patient showed clinical improvement, death occurred suddenly from an unrelated pulmonary infarct. Autopsy revealed viable cryptococci beneath the meninges

In all, a total of nineteen patients with acute and chronic cryptococcal meningitis have been treated with Actidione by intrathecal, intravenous and intramuscular routes by fifteen different investigating groups[11,53,58,65,124,141,156,170,185,186,238,254,393,413,470] Eleven of these patients died, no improvement was noted in two, some improvement was noted in four, and remissions were reported in two cases Wilson and Duryea's[470] patient and one of Carton's[58] (Case 5) appear to have had the best results with Actidione, both becoming asymptomatic However, a prolonged follow-up will be required to evaluate them properly Patients who improved on Actidione may well have represented examples of spontaneous remissions

In view of the synergistic action in vitro of polymyxin and Actidione against *C neoformans*,[58] this combination of drugs was administered to one patient with cryptococcal meningitis but without beneficial effects[254] Furthermore, daily intrathecal doses of hyaluronidase (Wydase) and streptodornase given in 50 T R U and 100,000 units, respectively, with both antibiotics, failed to halt the disease, spinal fluid cultures continued to be positive and the patient died one month after admission to hospital[254]

Actidione has been administered to humans by intramuscular, intravenous, intrathecal and intraventricular routes It has not caused abnormal hematologic changes *Toxic effects were occasional nausea and vomiting.* Actidione has been given intramuscularly in doses ranging from 10 mg. twice daily to 30 mg four times daily, intravenously in doses up to 180 mg. in twenty-four hours,

teriostatic to a variety of gram-positive and gram-negative bacteria. Although allicin inhibited *C. neoformans* at 1:100,000 (10 μg/ml.), experiments *in vivo* demonstrated no protection to mice infected by the intraperitoneal route.[220] LD_{50} of allicin was 60 mg./Kg. intravenously and 120 mg./Kg. subcutaneously.[125]

d. *Vaccine therapy:* The general impression that *C. neoformans* is poorly antigenic has given way to the present knowledge that thinly encapsulated strains are immunizing agents, and that relatively potent immune antiserum may be prepared in rabbits[293] (see Immunology, page 47). Immunologic studies of *C. neoformans* have indicated the presence of specific immunogenic polysaccharides which reside in the capsule of the cryptococcus, enabling serologic classification of the organism into Types A, B and C.[113,116] Positive skin reactions in patients with cryptococcal meningitis may appear as swelling and erythema upon testing with boiled aqueous extract of the organism, broth filtrate, and saline or acid treated suspensions.[29,58,81,210,236]

When crude, autogenous, heat-killed vaccines of *C. neoformans* were employed for therapeutic purposes in cryptococcosis, the results were disappointing. Shapiro and Neal[378] used autogenous vaccine intravenously for the therapy of cerebral cryptococcosis and observed no beneficial effect. Other routes of administration of vaccine were similarly unsuccessful in delaying the inevitable fatal outcome of the cerebral disease.[21,31,60,68,85,257,309,331,378,412] Perhaps some explanation of these poor results may be found in the relatively low levels of alpha₁ and gamma globulins measured in a patient with cryptococcal meningitis who was given typhoid vaccine intravenously to produce hyperthermia (see page 51). One patient with cryptococcal osteomyelitis, treated with autogenous vaccine, sulfadiazine, and iodides by Leopold[236] had almost complete bone regeneration, however, and six years later showed no evidence of cryptococcosis. Notwithstanding such experiences, purified, potent cryptococcal antigens should be studied for their usefulness in the therapy of cryptococcal meningitis, localized infection and in detection of skin hypersensitivity.

Desensitization procedure employed for hypersensitive individuals in the treatment of North American blastomycosis is not of value for cryptococcosis.

e. *Enzymes:* The enzymes streptokinase and streptodornase have been employed in the treatment of tuberculous meningitis in an effort to remove fibrinous blocks which prevent the free circulation of spinal fluid and thus permit streptomycin to diffuse more fully throughout the subarachnoid space and more quickly reach the tubercle bacilli.[419,484,502] Since the pathologic reaction in cryptococcal meningitis is somewhat similar, there is also a rational basis for the use of enzymes in this disease. Carton and Liebig[69] studied two enzymes and reported that streptokinase was not antagonistic in vitro to Actidione or polymyxin B, two antibiotics effective for *C. neoformans* (see Antibiotic therapy). The authors considered that combinations of antibiotics with streptokinase, streptodornase or hyaluronidase were feasible in human therapy as far as activity and toxicity were concerned. Hyaluronidase, moreover, was reported to destroy the cryptococcal capsule,[97] but this was not confirmed by Foley and Uzman[128] or by us (see Immunology). Consequently, Littman and Nathanson[254] adminis-

vitro against *C. neoformans*, protoanemonin was considered by Carton to be too toxic for intrathecal or systemic use.

Extracts of another plant of the Ranunculaceae family, *Coptis chinensis* (Huang-Lien), inhibited *C. neoformans* in a dilution of 1 80[69] This species of Ranunculaceae plant, long regarded by Chinese physicians as a panacea, grows extensively in the Chinese mainland, and in addition to possessing an active fungicidal substance, contains the alkaloids berberine, worenin and coptisin *

The Western red cedar, (*Thuja plicata D Don*), a tree highly resistant to decay, was found by Southam[398] to yield a hot-water extract which was inhibitory for *C. neoformans* at 115 mg. per cent (1150 μg/ml) Extracts were relatively nontoxic, since mice resisted intraperitoneal doses of 4 3 Gm./Kg and rabbits intravenous doses of 3 Gm /Kg Reports of further studies on this agent have not been encountered.

Tillandsia usneoides (Spanish moss) collected from trees of Charleston, South Carolina, yielded crude extracts containing inhibitory substances for *C. neoformans* at 400 to 1600 units/ml.[458]

Irving, Fontaine and Doolittle[199] discovered that the juice of tomato plants, "Bonny Best," "Rutgers," "Marglobe," "Pan America" and "Red Currant" (*Lycopersicon pimpinelliform*) contained an antibiotic which was strongly inhibitory to three plant-wilt pathogens of the *Fusarium* genus They named the substance "lycopersicin," but later changed it to "tomatin."[200] This was found to be markedly fungistatic and fungicidal in vitro to *Candida albicans* and three dermatophyte species[200] and was inhibitory for *C. neoformans* in concentration of 1 unit/ml[130] Kligman and Weidman,[220] however, found no fungistasis in vitro with a sample of crude tomatin Purified crystalline tomatine was prepared from crude tomatin by Fontaine and associates (cited by Ma and Fontaine[266]) The active substance, a glucosidal alkaloid, was called *tomatine* to distinguish it from crude *tomatin* referred to in earlier publications Ma and Fontaine[266] stated that the crystalline compound had low activity against bacteria but was active against fungi, and that activity of the crude tomatin was inhibited by rutin, a normal constituent of crude tomatin extracts The reversing effect of rutin was thought to account for differences in antibiotic properties of crude tomatin and crystalline tomatine

A crystalline protein called purothionin, extracted from wheat flour by Stuart and Harris,[410] exhibited bactericidal and fungicidal activity. *Saccharomyces cerevisiae* was inhibited at 1 200,000 (5 μg/ml) Minimal lethal dose for mice was 15 mg /Kg intraperitoneally[82]

A quinone-like compound extracted from the root of the plant *Plumbago europa* by Dulong d'Astafort[99] in 1829, and identified as 2-methyl-5-hydroxy-1, 4-naphthoquinone,[123] was demonstrated to be inhibitory to four species of pathogenic fungi in dilution of 1 50,000 (20 μg/ml)[354] Other naphthoquinones also effective in vitro were noted to be ineffective for cryptococcal infected mice.[220]

By extracting garlic cloves, *Allium sativum*, Cavallito and Bailey[64] isolated allicin ($C_6H_{10}OS_2$), a colorless oil with the odor of garlic, which was bac-

* Data secured through courtesy of *U S State Department*

Addendum

Ascospore production: In 1952, Wickerham and Burton[500] reported that some species of nonascospore-forming yeasts existed predominantly as haploid mating types, and that consequently ascospores were produced when sexually active strains of the opposite types were brought together under conditions favorable for sporulation. From these mating experiments, correlated with fermentation and assimilation tests, the authors proved that *Candida guilliermondii* and *Candida melibiosi* were one and the same species,[501] and they concluded that similar taxonomic and nomenclatural changes could be expected as the mating types of other imperfect species were found. Utilizing this technic, Benham[477] combined two strains of *C. neoformans* var. *innocuous* and two nonhuman strains of *C. neoformans* and reported ascospore formation similar to that described for the genus *Lipomyces*. She concluded that *Cryptococcus neoformans* and *Lipomyces starkeyi*,[495] the latter a ubiquitous lipid-producing soil yeast, might be one and the same organism. However, neither spore stains, nor virulence for mice, nor heat tolerance was reported for the latter organism. Our own studies of three strains of *Lipomyces starkeyi** and one of *Lipomyces lipoferus** revealed that they grew abundantly at 20°C on Sabouraud dextrose agar and Littman liver-spleen glucose blood agar, but unlike *C. neoformans* they failed to develop at 37°C. Furthermore, none of the three strains of *L. starkeyi* studied were pathogenic for white Swiss mice on intracerebral inoculation (see page 112). *It is doubtful, therefore, that C. neoformans and L. starkeyi are one* and the same organism, although some ecologic relationship may exist. Further studies on the mode of spore formation by both species as well as fermentation and assimilation tests are warranted.

Antifungal substances derived from actinomycetes: These substances have been found to exhibit a striking similarity to each other with respect to antifungal activity and elemental analysis and to possess a conjugated polyene chromophore group.[489,496,499] On the basis of differences in light absorption spectra, they have been subdivided into tetra-, penta-, hexa- and heptaenes.[499] Nystatin and rimocidin are examples of tetraenes, candicidin, candidin and ascosin represent heptaenes. Two additional polyenes, fungichromin and fungichromatin, have been described by Tytell and associates.[489,495]

Fungichromin,† derived from the actinomycete, *Streptomyces cellulosae*, is a pale yellow, crystalline compound containing no nitrogen, it is insoluble in water but soluble in organic solvents. In concentrated sulfuric acid, fungichromin produces a typical polyene reaction, yielding at first a violet color, which

* Obtained through the courtesy of Professor R. L. Starkey, Rutgers University, New Brunswick, N. J.

† Sharpe & Dohme, Inc., West Point, Pa.

tered daily intrathecal doses of 50 T.R.U. hyaluronidase (Wydase*) and 100,000 units streptodornase with Actidione and polymyxin to a patient with cryptococcal meningitis This treatment failed to reduce the number of viable cryptococci in the spinal fluid, to lessen the spinal fluid obstruction, or to influence the fatal outcome of the disease.

f *Effect of hormones* (see Addendum, page 150)

g *Symptomatic treatment:* Although specific medical therapy for cryptococcosis is not yet established, symptomatic treatment of the patient with cryptococcal meningitis should not be overlooked. The ambulatory patient with chronic meningitis and headaches not relieved by ordinary analgesics may require occasional lumbar puncture and withdrawal of spinal fluid. The patient should be cautioned against chronic constipation which may contribute to his symptoms The patient hospitalized with severe headache, abnormal neurologic manifestations, and perhaps in semi-coma, is greatly relieved and often restored to consciousness for several hours or days by spinal puncture and withdrawal of fluid This procedure may be continued as long as tolerated In one case, 100 lumbar punctures were performed safely over a period of seven months[378] In addition to providing temporary relief of pain, one may hope that the patient will be one of the fortunate few in whom a long period of remission is obtained A number of cases are on record in which patients survived for considerable periods of time though they received little therapy other than lumbar puncture[34,431] Remarkable elevations of intracranial pressure (700 mm water) occur in some patients At times, papilledema is so severe that vision is threatened If daily spinal puncture fails to control these manifestations, subtemporal decompression may be required When repeated spinal puncture becomes difficult to perform or painful to the patient, resort may be made to cisternal puncture

The ordinary opiates such as Demerol, morphine or Dilaudid usually offer the patient some relief Late in the course of the disease the patient may have difficulty swallowing, and tube feeding with high caloric diet may be necessary Although bowel and bladder disturbances are rare because of the infrequency of cord lesions, catheterization is usually necessary in the later stages of the disease

3 SUMMARY OF TREATMENT

In the absence of systemic or cerebral disease, the treatment of localized cryptococcosis is surgical When the disease is generalized and affects the central nervous system, medical therapy with Actidione (cycloheximide) or 2-hydroxystilbamidine appears to be the most reasonable at the present time Hyperthermia, administration of iodides, sulfonamides or immune serum, and vaccine therapy all appear to be without result Some hope is held that the newer fungicides or diamidine derivatives may prove more efficacious.

* Wyeth, Inc., Philadelphia, Pa

tablets and topical preparations appear to be well tolerated, with only minor side effects.

Newcomer and associates[490] administered nystatin (Mycostatin) orally, intramuscularly and intravenously to fourteen patients with disseminated coccidioidomycosis. Of ten patients who received oral treatment, only one was clinically improved by six months of treatment with doses as high as 12 grams daily. During treatment, blood levels of nystatin varied from 0 to 6 μg/ml Intramuscular injection of 200,000 units twice daily to two patients caused fever, chills, malaise, and pain and tenderness at the injection sites, necessitating discontinuance of therapy. Blood levels in these cases varied from 0 to 3 μg/ml. In one patient with miliary pulmonary coccidioidomycosis and subcutaneous abscesses, intravenous administration of 200,000 units nystatin hydrochloride initially caused fever, chills and generalized malaise, attributed to a Herxheimer-like reaction, which later subsided. Although clinical improvement did occur in this patient, treatment was discontinued because of the sclerosing effect of the preparation, even on the deep veins

Miscellaneous compounds: Renzetti and Feldman[492] reported successful treatment with Daraprim®* in the case of a 26 year old white male veteran who suffered fever, chills and night sweats and had generalized cryptococcosis and pulmonary tuberculosis. C *neoformans* was demonstrated in the skin on numerous occasions, and was recovered from the blood stream but not from the spinal fluid The control of both diseases was credited to treatment with streptomycin, isoniazid, Daraprim and sulfadiazine. Daraprim, an antimalarial nucleic acid antagonist, with the formula 2,4-diamino-5-p-chlorophenyl-6-ethyl pyrimidine, was administered daily with sulfadiazine in doses of 50 mg, then decreased to 25 mg After five months of therapy a skin biopsy still revealed cryptococci, but two months later all skin lesions had healed and the patient was discharged shortly thereafter A follow-up thirteen months later revealed him to be in good health. Despite this favorable report, our own experiments in vitro with alcoholic solutions of Daraprim in 2, 5 and 10 μg/ml. concentrations of the drug in Sabouraud dextrose broth failed to show an inhibitory effect upon the growth of two strains of C *neoformans* isolated from cases of cryptococcal meningitis Unless Daraprim is selectively concentrated in infected tissues, a 70 Kg individual who receives one 25 mg tablet daily would have a tissue level of only 0 35 μg/ml., much too low to provide any beneficial effect in cryptococcal infections (see "Id[illegible]

In a study of [illegible]
ciates[494] *confirmed* [illegible]
found to synthesize the pyrimidine part of the thiamine molecule (2-methyl-5-ethoxymethyl-6-amino-pyrimidine) but not the thiazole part (4-methyl-5-thiazoleethanol), and to combine readily these two moieties to form thiamine [illegible]

[illegible] the following compounds

* Trademark, brand pyrimethamine, Burroughs Wellcome

then turns to blue. The compound inhibited the growth of *Blastomyces dermatitidis* and *Candida albicans* in concentrations, respectively, of 0.78 μg/ml. and 6.25 μg/ml. Toxicity for mice was reported to be 16.4 mg./Kg. intraperitoneally, and over 1000 mg./Kg orally. *Fungichromatin*, similar to fungichromin in chemical and antimicrobial properties, was also recovered from an actinomycete.[498]

Mycoticin, isolated by Burke and associates[478] from *Streptomyces ruber*, is a yellow, crystalline, antifungal antibiotic that fluoresces and also deteriorates under the influence of ultra-violet light. This antibiotic inhibits the growth of *C. neoformans* at 6 to 8 μg/ml., and *Coccidioides immitis, Blastomyces dermatitids, and Sporotrichum schenckii* at 1 to 4 μg/ml. Mycoticin has a characteristic ultra-violet spectrum, contains no nitrogen, sulfur or halogens, and is soluble in lower alcohols and glycols. Although it loses its activity at room temperature, it can be preserved in vacuo in the dark for prolonged periods of time without loss of potency. The toxicity of the compound is significant, for the LD_{50} for mice is 10–20 mg /Kg. on intraperitoneal injection Subcutaneous administration results in tissue necrosis and sloughing. Its parenteral use in humans, therefore, would be prohibited unless nontoxic derivatives were developed

Nystatin* (Mycostatin®), an antifungal antibiotic without antibacterial activity, is a pale yellow microcrystalline powder which has a potency of 2500 to 3000 units per milligram against *Candida albicans*. It is an amphoteric substance with the formula $C_{46}H_{83}NO_{18}$, insoluble in water but soluble in propylene glycol and methanol, and containing no halogen, sulfur, methoxyl, phenolic or acetyl groups. It is optically active and possesses an ultra-violet absorption spectrum characteristic of a conjugated tetraene. Nystatin is quite unstable in solution and is rapidly inactivated by hydrogen and hydroxyl ions, heat, light and oxygen, however, dry solids stored in sealed containers under refrigeration maintain their activity for longer than six months. Blood reduces its activity A water solution of nystatin loses 37 5 per cent of its potency when held for six hours, while plasma nystatin solutions lose 50 per cent However, tablets of the compound are reported to maintain full potency when kept dry at 40°C Assay in vitro consists of a disc-agar diffusion procedure against a strain of *C albicans*, lower values being obtained by broth assay A satisfactory chemical method of assay has not yet been developed A biologic method similar to that of Tarbet and Sternberg[420] has been employed for the determination of blood levels (see page 140)

Efforts to treat cryptococcosis with nystatin by the parenteral route have been unsuccessful (see page 139) In addition, results with nystatin have been disappointing in seven other cases of cryptococcal meningitis Several different investigators noted no improvement in two patients who received nystatin orally and 5 patients who were treated intramuscularly * Significant levels of nystatin apparently do not appear in the spinal fluid The parenteral toxicity of nystatin is considerable, judging from the manufacturer's statement that "severe local reactions are often observed following the intramuscular injection of Mycostatin and its general use is not advised except in desperate cases " Nevertheless, oral

* Data drawn from bulletin "Mycostatin,' August, 1955, with the permission of E R Squibb & Sons, Inc.

given an extensive trial at the Armed Forces Institute of Pathology. In addition to yielding the most photogenic preparations for black-and-white photography, it was unsurpassed for easy detection of small numbers of fungi and in certain cases it was the only effective fungus stain for demonstrating *Mucor*.

Immunology: The preparation from *C. neoformans* of a complement-fixing antigen for diagnostic use was described by Fischer and Labzoffsky.[501] Cryptococcal cells, cultivated for 7 days at 37°C on Sabouraud agar and harvested with buffered saline, were treated with pyridine for two hours at room temperature, washed three times with buffered isotonic saline, resuspended and exposed to ultrasonic vibration for 10 minutes. Although crude, the antigen was heat stable, not anticomplementary, and appeared to be both sensitive and specific. When combined with antigens of three strains of *C. neoformans* bearing capsules of differing thicknesses, immune anticryptococcal rabbit serum titrated to 1:128. Cross reactions did not occur with immune *Histoplasma* or *Blastomyces* sera. Evaluation of this new antigen for diagnostic purposes in human disease was not accomplished because of the unavailability of sera from clinical cases (see Serology in Diagnosis, page 48).

Involvement of other organs: Another instance of cardiovascular cryptococcosis has been recorded in the case of a patient with rheumatic heart disease admitted for cardiac surgery to the Clinical Center of the National Institutes of Health. On physical examination the patient was found to be febrile and to have petechiae characteristic of subacute bacterial endocarditis. Culture of blood, urine, and spinal fluid repeatedly yielded *C. neoformans*. At autopsy a large vegetation containing cryptococci was found on the posterior leaflet of the mitral valve and several small vegetations were present on the cusps of the aortic valve. Microscopic examination revealed widespread dissemination of the fungus* (see page 35).

Source of infection and portal of entry: The suspicion that the alimentary tract could serve as a portal of entry of *C. neoformans* (see pages 6 and 39) was confirmed experimentally by Takos,[504] who produced systemic and cerebral cryptococcosis in 2 of 3 marmoset monkeys by feeding 50 to 150 million cells of a culture isolated from a fatal human case. All 3 monkeys became ill within 24 hours after ingesting cryptococci, and one died within four days with paralytic ileus involving the entire small bowel. Histologic examination of the intestine failed to reveal evidence of invasion or inflammation. This phenomenon would suggest the presence of a cryptococcal enterotoxin (see page 48 for endotoxic substance from *C. neoformans*). The remaining 2 monkeys died within 19 and 32 days with focalized lesions of the brain, mesenteric lymph nodes and myocardium. Cultures from the meninges, brain, spinal cord, large and small intestine, liver, and spleen were positive for the organism. The study indicates that *C. neoformans* can survive exposure to gastric juice of the marmoset and retain undiminished virulence, even after several weeks residence in the monkey's intestinal tract. Experimental demonstration of the digestive tract as a portal of entry of *C. neoformans* may assume considerable epidemiologic importance in view of the fact that virulent strains of the organism have been isolated from fruit,[359 359] raw milk,[56,213 324 386] soil[105 107] and pigeon excreta.[109 110]

* Personal communication from Drs. T. A. Lombardo, A. S. Rabson, and H. T. Dodge.

	Inhibiting concentration		Inhibiting concentration
methyl-5-nitro-2-furoate	5 μg/ml	pseudomethyl acetylacrylate	25 μg/ml
dithiocyanoacetanilide	5 μg/ml	8-hydroxyquinoline	5 μg/ml
hexachlorophene	5 μg/ml	dimethyl dichlorosuccinate	5 μg/ml
Synkamin (Vitamin K derivative)	5 μg/ml	cetyl dimethyl ethyl ammonium bromide	5 μg/ml
biacetyl	25 μg/ml		
eschridine	25 μg/ml	cetyl trimethyl ammonium bromide	5 μg/ml

The first seven compounds listed above, with LD_{50} values ranging from 18 to 1320 mg./Kg., failed to influence the fatal course of mice infected intracerebrally with *C neoformans.*

Chloroquinaldol®,* used with success for several years as an intestinal antiseptic in Europe and South America under the name "Siosteran," has been found by Littman[487] to exert a pronounced inhibitory effect in vitro on *C neoformans* and other systemic pathogenic fungi The growth of three strains of *C. neoformans* isolated from cases of cryptococcal meningitis was inhibited by 4 to 10 μg/ml of the compound in Sabouraud dextrose broth, in both the presence and absence of 10 per cent horse serum Chlorquinaldol, a crystalline yellowish powder with the formula 5,7-dichloro-8-hydroxyquinaldine, apparently is well tolerated orally and is of low toxicity [488]

Animal	Rat	Mouse	Guinea pig	Dog
LD_{50}	3100 mg /Kg	500 mg /Kg	50 mg /Kg	over 1000 mg /Kg

Blood levels and animal protection tests are not yet reported. The effectiveness in vitro for *C neoformans* of Chlorquinaldol and related compounds such as 8-hydroxyquinoline[494] merits further study of these and other substituted quinolines as therapeutic agents for cryptococcosis and other mycotic diseases

Effect of hormones: Mankowski[488] *administered estradiol intramuscularly* to cryptococcal-infected white mice and noted that, in contrast to a control group, it shortened their life span. The mechanism of the action was unknown, although it was noted that neutrophil counts were consistently lowered by administration of the hormone Konigsbauer[485] noted that cortisone in large doses (40 mg /Kg) injected subcutaneously into cryptococcal-infected white rats accelerated the spread and intensity of the infection Cortisone, when administered to *Hormodendrum*-infected rats, depressed the proliferation of connective tissue and reduced the numbers of leukocytes and lymphocytes within the inflamed granulation tissue There is growing suspicion that administration of cortisone to patients with cryptococcosis accelerates the disease [232 216]

Histopathologic diagnosis: Although some current literature suggests that improved staining technics for infectious agents in tissue could replace culture methods, the experience of Starr and associates[496] at the Mayo Clinic indicates that staining methods must still remain supplementary to cultural procedures Grocott[491] applied Gomori's methenamine-silver nitrate technic for demonstration of glycogen and mucin to the study of fungi. He found the method to yield highly photogenic black-and-white preparations in which *fungal elements were* sharply delineated against an unstained background The Gomori stain was

* Trademark, Compound VR-764, Geigy Pharmaceuticals, New York

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* Trademark, Compound VR-764, Geigy Pharmaceuticals, New York.

4 LITTMAN OXGALL AGAR[156, 381, 384] (Difco Laboratories)

Peptone (Difco)	10 Gm
Dextrose	10 Gm
Oxgall, dehydrated (Difco)	15 Gm
Agar	20 Gm
Crystal violet	0 01 Gm
Distilled water	1000 ml
pH	7 0

5. LITTMAN MEDIUM[156, 381, 384] (Baltimore Biological Laboratory, Inc, Baltimore, Md.)

Oxgall	15 Gm
Gelysate and polypeptone (BBL)	*10 Gm*
Dextrose	10 Gm
Agar	16 Gm
Crystal violet	0 01 Gm
Distilled water	1000 ml
pH	7 0

Directions for formulas 4 and 5

a Dissolve peptone, dextrose and oxgall in distilled water.
b Adjust pH to 7 0
c Add crystal violet, agar, and melt If dehydrated commercial media are employed, suspend and dissolve the recommended quantities in distilled water.
d Autoclave 15 lbs (121°C) for 15 minutes For Littman Medium, use 12 lbs (118°C) for 15 minutes.
e Cool the sterile medium to 45–50°C Aseptically add, to each liter of medium, 30,000–50,000 μg streptomy*cin contained in 10 ml sterile distilled water*
f Dispense 25–30 ml amounts in sterile petri dishes and allow 6–8 hrs at room temperature before inoculation (see page 102)
g Plates are incubated at 20° to 25°C. Do not incubate at 37°C

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6 BRAIN-HEART INFUSION BLOOD AGAR[174]

Calf brain, infusion from	200 ml
Beef heart, infusion from	250 ml
Peptone (gelysate, BBL, or proteose-peptone, Difco)	10 Gm
Dextrose	2 Gm
NaCl	5 Gm
Na_2HPO_4	2 5 Gm
Agar	20 Gm
Distilled water	1000 ml
pH	7 4

a Infuse brain and heart separately as follows

1 Remove fat from tissues, grind
2 Infuse ground heart overnight in water at 4–6°C
3 Heat to 45–50°C for one hour
4 Boil for ½ hour, cool without stirring to 10–15°C, remove fat as solid coagulum
5 Filter through glass wool.

b Combine infusions, make up to 1000 ml with distilled water and add peptone, dextrose and salts
c Adjust to pH 7 4, add agar and melt If dehydrated commercial medium is employed, suspend and dissolve the recommended quantity in distilled water
d Autoclave at 15 lbs (121°C) for 15 minutes
e Cool the sterile medium to 45–50°C Aseptically add, to each liter of medium, 20,000 units penicillin and 50,000 units streptomycin contained in 10 ml sterile distilled water and add 10 per cent whole animal or human bank blood
f Dispense in sterile petri dishes and tubes (see page 102)

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7 LITTMAN LIVER-SPLEEN GLUCOSE BLOOD AGAR[382]

Beef spleen, infusion from	500 ml
Beef liver, infusion from	500 ml
Thiotone (BBL)	10 Gm
Dextrose	10 Gm
Agar	20 Gm
pH	7 7

a Infuse beef spleen and beef liver separately as follows

1 Remove fat from liver, strip capsule from spleen, grind separately
2 Infuse 1000 grams ground substance with 2000 ml distilled water at 50°C for one hour with occasional agitation

(cont'd, next page)

Appendix

1 SABOURAUD DEXTROSE AGAR (Modified from Sabouraud[183])

Neopeptone (Difco) or polypeptone (BBL)	10 Gm
Dextrose	40 Gm
Agar	20 Gm
Distilled water	1000 ml
pH	7.0

a. Dissolve ingredients in distilled water and melt agar with heat
b Autoclave at 12 lbs (118°C) for 10 minutes
c Cool the sterile medium to 45–50°C *Aseptically add 20,000 units penicillin* and 50,000 units streptomycin contained in 10 ml sterile distilled water to each liter of medium
d. *Dispense in sterile petri dishes or tubes* and slant
e For isolation of *C. neoformans* do not use Sabouraud dextrose agar that contains Actidione since the organism is sensitive to this antibiotic Thiamine, in concentration of 5 mg per liter, is of value in this medium for stimulation of growth of dermatophytes, especially the faviform Trichophytons
f. Incorporation of penicillin and streptomycin eliminates the need for acidity (pH 5 6) as a bacterial inhibitor. Pathogenic fungi grow well at neutral or slightly alkaline reaction
g The medium may be used without contained antibiotics *(see page 101)*, for isolation of *Nocardia* and other antibiotic-sensitive species and for maintenance of stock cultures

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2 MYCOLOGICAL AGAR (Difco Laboratories)

Soytone (Difco)	10 Gm
Dextrose	10 Gm
Agar	15 Gm
Distilled water	1000 ml
pH	7 0

3 MYCOPHIL AGAR (Baltimore Biological Laboratory, Inc)

Phytone (BBL)	10 Gm
Dextrose	10 Gm
Agar	16 Gm
Distilled water	1000 ml
pH	7 0

Directions for formulas 2 and 3

a Dissolve ingredients in distilled water, add agar and melt with heat If the dehydrated commercial media are employed, suspend and dissolve the recommended quantity in distilled water
b Autoclave at 15 lbs (121°C) for 15 minutes, for Mycophil agar use 12 lbs (118°C) for 15 minutes
c Cool the sterile medium to 45–50°C Aseptically add 20,000 units penicillin and 50,000 units streptomycin contained in 10 ml sterile distilled water to each liter of medium
d Pour into sterile petri dishes or tubes
e *Both culture media may be used* without antibiotics for stock cultures (see page 101) and for demonstration of chromogenesis

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NOTE Culture media for diagnostic medical mycology may be obtained in the dehydrated form from

1 Difco Laboratories, Detroit, Michigan (Formulas 1, 2, 4, 6, 8, 10, 12)

2 Baltimore Biological Laboratory, Inc (BBL), Baltimore, Maryland (Formulas 1, 3, 5, 6).

9. CARBON ASSIMILATION AGAR (AUXANOGRAPHIC) FOR YEASTS[34]

$(NH_4)_2SO_4$	5 Gm
KH_2PO_4	1 Gm
$MgSO_4 \cdot 7H_2O$	0 5 Gm
Washed agar	20 Gm
Distilled water	1000 ml

a Prepare washed agar by soaking 20 grams minced agar in 1000 ml distilled water for 8 days at room temperature, followed by several rinses in fresh distilled water

b Dissolve ingredients in distilled water and melt agar with heat

c. Autoclave at 110°C for 15 minutes

d Cool the sterile medium to 45–50°C, seed with a viable suspension of the yeast under test and dispense in sterile petri dishes Allow the surface of the agar to dry before planting carbohydrate test compounds In the event of uncertain results (see page 105), one drop of yeast extract or B vitamin solution may be added to each petri plate of medium

10. CARBON ASSIMILATION BROTH (YEAST NITROGEN BASE)[34] (Difco Laboratories)

Nitrogen sources	
Ammonium Sulfate	5 Gm
Asparagine	None
Carbon source	
Dextrose	None
Amino acids	
l-Histidine Monohydrochloride	10 mg
dl-Methionine	20 mg
dl-Tryptophane	20 mg
Vitamins	
Biotin	2 µg
Calcium Pantothenate	400 µg
Folic Acid	2 µg
Inositol	2000 µg
Niacin	400 µg
p-Aminobenzoic Acid	200 µg
Pyridoxine Hydrochloride	400 µg
Riboflavin	200 µg
Thiamine Hydrochloride	400 µg
Compounds supplying trace elements	
Boric Acid	500 µg
Copper Sulfate	40 µg
Potassium Iodide	100 µg
Ferric Chloride	200 µg
Manganese Sulfate	400 µg
Sodium Molybdate	200 µg
Zinc Sulfate	400 µg
Salts	
Potassium Phosphate Monobasic	1 Gm
Magnesium Sulfate	0 5 Gm
Sodium Chloride	0 1 Gm
Calcium Chloride	0 1 Gm
Distilled water	1000 ml
pH	4 5

a Dissolve above ingredients in 100 ml distilled water to produce 10 × strength, adjust to pH 4 5 Sterilize by Seitz or Berkefeld filtration

b Dissolve 0 5 gram dextrose or other test carbohydrate in 90 ml distilled water Sterilize by Seitz or Berkefeld filtration

c Store solutions in refrigerator

d The final liquid medium is prepared by pipetting aseptically 0 5 ml of sterile basal medium into 4 5 ml sterile carbohydrate solution and mixing

e Inoculum is prepared by growing the yeast culture on yeast and malt extract agar slants This medium contains 3 Gm powdered yeast extract, 3 Gm malt extract, 5 Gm of peptone, 10 Gm of dextrose and 20 Gm of agar in one liter of distilled water, pH lies between 5 0–6 0, depending upon the batch of ingredients used In order to adapt the yeast cells to grow in a synthetic medium and to reduce the carbohydrate reserve in the cells, cultures are grown in carbon assimilation broth with 0 1% dextrose, then with no dextrose at all The inoculum consists of one drop of yeast suspension in this last synthetic medium The inoculum should not produce turbidity in the carbon assimilation broth

f For rapid and latent results examine tubes after 7 and 24 days at 25°C, first shaking to re-suspend growth

g Read test by placing the tube against a white card bearing India ink lines approximately ¾ mm thick

h The test is positive if sufficient turbidity appears to make the lines appear as diffuse broad bands or to obliterate them (see page 106).

7. **LITTMAN LIVER-SPLEEN GLUCOSE BLOOD AGAR** (*cont'd*)

3 Heat at 80°C for 5 minutes
4 Filter hot through three layers consisting of a towel, four thicknesses filter paper and moist gauze-wrapped cotton, wrung dry.
5 Cool to 10–15°C to harden fat, remove, refilter through filter paper
6 Add 10 grams thiotone to each liter of infusion, adjust to pH 7.7 with NaOH
7. Autoclave at 15 lbs. (121°C) for 15 minutes
8 Cool, allow suspended particles to settle, refilter. This may be re-autoclaved and stored for future use.

b Combine infusions, add dextrose, re-adjust to pH 7.7.
c. Add agar, melt and autoclave at 10 lbs. (116°C) for 10 minutes
d Cool sterile medium to 45–50°C Aseptically add, to each liter of medium, 20,000 units penicillin and 50,000 units streptomycin contained in 10 ml sterile distilled water and add 10 per cent sterile human bank blood.
e *Dispense in sterile petri dishes and* tubes (see page 103).

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8 **YEAST MORPHOLOGY AGAR**[103] (Difco Laboratories)

Nitrogen sources	
Ammonium Sulfate	3 5 Gm
Asparagine	1 5 Gm
Carbon source	
Dextrose	10 0 Gm
Amino acids	
l-Histidine Monohydrochloride	10 mg
dl-Methionine	20 mg
dl-Tryptophane	20 mg
Vitamins	
Biotin	2 μg
Calcium Pantothenate	400 μg
Folic Acid	2 μg
Inositol	2000 μg
Niacin	400 μg
p-Aminobenzoic Acid	200 μg
Pyridoxine Hydrochloride	400 μg
Riboflavin	200 μg
Thiamine Hydrochloride	400 μg
Compounds supplying trace elements	
Boric Acid	500 μg
Copper Sulfate	40 μg
Potassium Iodide	100 μg
Ferric Chloride	200 μg
Manganese Sulfate	400 μg
Sodium Molybdate	200 μg
Zinc Sulfate	400 μg
Salts	
Potassium Phosphate Monobasic	1 Gm
Magnesium Sulfate	0 5 Gm
Sodium Chloride	0 1 Gm
Calcium Chloride	0 1 Gm
Agar	18 Gm
Distilled water	1000 ml
pH	4 5

a Dissolve ingredients in distilled water, adjust pH, add agar, melt with heat If dehydrated commercial medium is employed, suspend and dissolve the recommended quantity in distilled water
b Autoclave at 15 lbs (121°C) for 15 minutes.
c Cool to 45–50°C and pour plates very thin to depth of *1 5 mm*, *allow plates* to stand at room temperature 24 hours, for dry surface
d. Inoculate by streaking lightly across the plate (from positions 10 o'clock to 2 o'clock) and two point inoculations *at 4 and 8 o'clock*
e Cover one of the point inoculations and the center of the streak with alcohol-sterilized, flamed, cooled coverslips (Dalmau preparation, consisting of aerobic and anaerobic areas, for morphologic study of yeasts)
f Incubate 6–7 days at 25°C for hyphae of filamentous yeasts.
g For colonial differentiation of *C neoformans* from *C neoformans* var *innocuous*, prepare giant colonies (see page 102) on sterile medium poured 25–30 ml to each petri dish.

13 HENRICI'S VEGETABLE JUICE SPORULATION AGAR FOR YEASTS, MODIFIED[58]

Mixed vegetable juice	500 ml
Compressed yeast cake	10 Gm
Agar	20 Gm
Distilled water	500 ml
pH	6 8

a Contents of one can of mixed juice from eight vegetables* (1 pint, 2 oz), is adjusted to pH 6 8 with potassium hydroxide.
b Disperse one-half cake (10 Gm) of compressed yeast in the vegetable juice and steam the mixture for ten minutes to kill the yeast cells and liberate their acids
c Readjust to pH 6 8 and add an equal volume of hot distilled water containing 4 per cent melted agar, mix
d Dispense in tubes, autoclave at 15 lbs (121°C) for 15 minutes
e Remelt and slant tubes before use. Incubate cultures at 25°C (see page 103)

* Commercially available as "V-8," Standard Brands, Terre Haute, Indiana, U S A or other brands

14 CARROT INFUSION SPORULATION AGAR FOR YEASTS[71, 87]

Chopped carrots	150 Gm
Agar	15 Gm
$CaSO_4$ (anhydrous)	3 5 Gm
Tap water	1000 ml

a Infuse finely chopped carrots in distilled water overnight in refrigerator
b Heat to 60°C for one hour, filter through paper
c To the filtrate add agar, melt with heat, add $CaSO_4$, mix thoroughly.
d Dispense in tubes, autoclave at 15 lbs (121°C) for 15 minutes
e Remelt and slant tubes before use (see page 103)

15 SYNTHETIC AGAR MEDIUM FOR DETECTION OF EXTRACELLULAR STARCH PRODUCTION BY YEASTS[78]

$(NH_4)_2SO_4$	1 Gm
$MgSO_4$	0 5 Gm
KH_2PO_4	1 Gm
Dextrose	10 Gm
Thiamine	200 μg
Agar	25 Gm
Distilled water	1000 ml
pH	4 5

a Dissolve ingredients in distilled water
b Adjust pH to 4 5 with dilute HCl
c Add agar, melt and autoclave at 110°C for 15 minutes
d Cool and dispense in sterile petri dishes (see page 107)

11. NITROGEN ASSIMILATION AGAR (AUXANOGRAPHIC) FOR YEASTS[304]

KH_2PO_4	1 Gm
$MgSO_4 \cdot 7H_2O$	0 5 Gm.
Dextrose	20 Gm
Washed agar	20 Gm
Distilled water	1000 ml

a Prepare washed agar by soaking 20 grams minced agar in 1000 ml distilled water for 8 days at room temperature, followed by several rinses in fresh distilled water

b Dissolve ingredients in distilled water and melt agar with heat

c Autoclave at 110°C for 15 minutes

d Cool the sterile medium to 45–50°C, seed with a viable suspension of the yeast under test and dispense in sterile petri dishes Allow the surface of the agar to dry before planting KNO_3 and peptone (see page 106).

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12. NITROGEN ASSIMILATION BROTH (YEAST CARBON BASE)[407] (Difco Laboratories)

Nitrogen sources	
Ammonium Sulfate	None
Asparagine	None
Carbon source	
Dextrose	10 Gm
Amino acids	
l-Histidine Monohydrochloride	1 mg
dl-Methionine	2 mg
dl-Tryptophane	2 mg
Vitamins	
Biotin	2 μg
Calcium Pantothenate	400 μg
Folic Acid	2 μg
Inositol	2000 μg
Niacin	400 μg
p-Aminobenzoic Acid	200 μg
Pyridoxine Hydrochloride	400 μg
Riboflavin	200 μg
Thiamine Hydrochloride	400 μg
Compounds supplying trace elements	
Boric Acid	500 μg
Copper Sulfate	40 μg
Potassium Iodide	100 μg
Ferric Chloride	200 μg
Manganese Sulfate	400 μg
Sodium Molybate	200 μg
Zinc Sulfate	400 μg
Salts	
Potassium Phosphate Monobasic	1 Gm
Magnesium Sulfate	0 5 Gm
Sodium Chloride	0 1 Gm
Calcium Chloride	0 1 Gm
Distilled water	1000 ml
pH	4 5

a. Dissolve above ingredients in 100 ml distilled water to produce 10 × strength, adjust to pH 4 5 Sterilize by Seitz or Berkefeld filtration

b Dissolve 0 078 gram KNO_3 in 90 ml distilled water Filter sterilize.

c The final medium is prepared by aseptically adding 4 5 ml sterile nitrate solution to 0 5 ml sterile basal medium and mixing

d The inoculum is prepared by growing the yeast on slants of yeast extract agar This medium contains 3 Gm powdered yeast extract, 5 Gm peptone, 10 gm dextrose and 20 Gm agar in one liter distilled water, pH 6 8–7 0

e Reserves of nitrogen carried by yeasts which have been grown on protein-rich media are depleted by two serial transfers on the synthetic nitrogen assimilation broth containing KNO_3 After 7 days' incubation at 25°C, the culture is shaken, one loopful transferred to a second tube of the same basal medium containing potassium nitrate This is done to eliminate the carry-over of peptone nitrogen A positive test in this second culture within 7 days indicates that the organism assimilates potassium nitrate (see page 107)

17. GRIDLEY FUNGUS STAIN[101]

USE This method is one of the best for the demonstration of both fungal spores and hyphae. Since this technic stains relatively few tissue elements, it is excellent for use in screening tissues for the presence of fungi other than *Actinomyces,** *Nocardia** and *Mucor.*†

PRINCIPLE. Since the periodic acid-Schiff stain does not color fungal hyphae in tissue as well as it stains spores, Gridley developed a new stain for fungi which employs the Feulgen reagent in combination with Gomori's aldehyde-fuchsin stain for elastic tissue fibers The Gridley stain possesses the advantage of staining spores and hyphae in tissue equally well Gomori's aldehyde-fuchsin stain causes hyphae to assume a deep blue color The problem of positive tissue elements with the periodic acid-Schiff reaction is reduced by oxidation with chromic acid instead of periodic acid

Two main steps are involved in the Gridley staining process the tissue is first oxidized with chromic acid and permitted to react with Coleman's leucofuchsin reagent rather than Schiff's, then it is placed in Gomori's aldehyde-fuchsin stain Counterstaining is with Metanil yellow because of its pleasantly contrasting color

TECHNIC

a Cut well-fixed, paraffin-imbedded tissues at 6 microns thickness and affix to microslides with albumin or gelatin, dry
b Deparaffinize to distilled water
c Oxidize in 4 per cent chromic acid for 1 hour
d Wash in running water for 5 minutes
e Place in Coleman's preparation of Feulgen reagent for 15 minutes

(Dissolve 1 Gm of basic fuchsin in 200 ml of boiling water, filter, cool, and add 2 Gm. of potassium metabisulfite ($K_2S_2O_5$) and 10 ml of N/1 hydrochloric acid Permit bleaching for 24 hours, then add 0 5 Gm. of activated carbon (Norit), shake for one minute and filter through coarse filter paper The filtrate should be colorless)

f Rinse in 3 changes of sulfurous-acid solution, 2 minutes for each rinse

10 per cent sodium metabisulfite	6 ml
N/1 hydrochloric acid	5 ml
Distilled water	100 ml

g Wash for 15 minutes in running water
h Place in aldehyde-fuchsin solution for 15 to 20 minutes

Basic fuchsin	1 Gm
70 per cent ethyl alcohol	200 ml
Paraldehyde	2 ml
Hydrochloric acid, concentrated	2 ml

Hold at room temperature for 3 days until solution turns deep blue, then refrigerate

i Rinse off excess stain in 95 per cent ethyl alcohol
j Wash well in water
k Counterstain in Metanil yellow solution for 2 to 5 minutes

Metanil yellow	0 25 Gm
Distilled water	100 0 ml
Glacial acetic acid	0 25 ml

l Wash in water
m Dehydrate, clear and mount in Permount

RESULTS Hyphae, deep blue Conidia, deep rose to purple Background, yellow. Elastic tissue and mucin also stain deep blue

* *Actinomyces* and *Nocardia* are demonstrated best by Gram's stain (Appendix formula 19)

†*Mucor* is stained more effectively by hematoxylin and by Gomori's silver nitrate technic (Appendix formula 20)

16 HOTCHKISS-McMANUS STAIN, PERIODIC ACID-SCHIFF TECHNIC[317]

USE· This stain is extremely useful for the demonstration of histologic structures containing carbohydrates with the 1,2-glycol grouping in the unsubstituted form or the equivalent chemical structures in which OH groups are replaced by amino or alkylamino groups Since the cell walls of most fungal species contain similar substances, mycelial and yeastlike fungi stain brilliantly with this histochemical method However, tissue elements are also stained Species of *Actinomyces* and *Nocardia* are not demonstrated satisfactorily by this technic, but are stained well by the Brown and Brenn Gram stain (Appendix formula 19)

PRINCIPLE· Tissue sections are first oxidized with periodic acid to convert the 1,2-glycol groupings in the fungal cells into aldehydes When Schiff's reagent is added to react with the aldehyde groups, recolorization of the reagent occurs to form compounds of varying shades of red and magenta, thereby coloring the cell walls of the fungi By counterstaining with Light Green (the modification of Kligman and associates[177]) some of the positive tissue elements are overstained and the fungi present are seen to better advantage

TECHNIC:

a. Cut well-fixed, paraffin-imbedded tissues at 6 microns thickness and affix to microslides with albumin or gelatin; dry. (Skin scrapings and fresh smears fixed in 95% alcohol may also be stained by this technic, consult reference).
b Deparaffinize to distilled water
c Oxidize in 1% periodic acid for 5 minutes.
d Wash in running tap water for 15 minutes
e. Place in Schiff's reagent for 10–15 minutes (Prepare the reagent by bringing to boil 1 Gm of basic fuchsin in 200 ml distilled water Upon cooling to 50°C, filter the solution and add 20 ml. N/1 hydrochloric acid Cool the solution further and add 1 Gm anhydrous sodium bisulfite Keep the solution in the dark for 48 hours until it becomes straw-colored, it is then ready for use)
f Transfer directly to two changes of either of the following solutions, allowing 5 minutes for each change

(1)	10 per cent potassium metabisulfite ($K_2S_2O_5$)	5 ml
	N/1 hydrochloric acid	5 ml
	Distilled water	100 ml
(2)	Thionyl chloride	5 ml
	Distilled water	100 ml

g Wash in running water for 10 minutes
h Counterstain briefly with Light Green
i Dehydrate, clear and mount

RESULTS Fungal spores stain bright red to shades of magenta, cell walls of hyphae are stained and appear as hollow tubes Background light green

19 BROWN AND BRENN GRAM STAIN (Gridley's Manual)

USE Most of the fungi are demonstrable in tissue sections as Gram-positive organisms In our opinion, this stain is the one of choice for *Actinomyces* and *Nocardia*, but it is unsuitable for mycelated and yeastlike fungi which are demonstrated more clearly by the Gridley and Hotchkiss-McManus stains *

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* Kade and Kaplan[83] recommended this Gram stain as the one of choice for *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Candida albicans* as well as for *Actinomyces bovis* and *Nocardia asteroides*

TECHNIC.

a Cut paraffin sections of any well fixed tissue at 4 to 6 microns thickness and affix to microslides with albumin or gelatin Dry and deparaffinize to distilled water
b Place slide on staining rack and cover with 1 ml. one per cent aqueous crystal violet and 5 drops 5 per cent sodium bicarbonate for 2 minutes; agitate gently.
c Wash in water
d Flood slide with Gram's iodine solution for 30 seconds.

Iodine	1 0 Gm.
Potassium iodide	2 0 Gm
Distilled water	300 0 ml

e Rinse in water and blot with filter paper
f Decolorize by dropping on the slide a mixture of equal parts acetone and ether until color ceases to appear
g Stain with basic fuchsin solution for 5 minutes

Saturated aqueous solution of basic fuchsin	0 1 ml
Distilled water	100 0 ml

h Wash in water and blot gently
i Transfer to acetone
j Decolorize in picric acid-acetone solution until sections are yellowish pink

Picric acid	0 1 Gm
Acetone	100 0 ml

k Rinse in acetone, then in equal parts acetone and xylene
l. Clear in xylene and mount in Permount

RESULTS Fungi stain varying shades of blue and violet, many unstained forms

18. MAYER'S MUCICARMINE STAIN (Gridley's "Manual of Special Staining Technics" employed at the Armed Forces Institute of Pathology)

USE. This method is a traditional one for the demonstration of epithelial mucin, however, it also stains the mucinous capsule and the cell wall of the Cryptococcus an intense pink to red color We have not encountered other yeastlike fungi of similar morphology that stain as intensely or as consistently with mucicarmine*, hence this method is of value in the differential diagnosis of *C neoformans* from other non-mycelated yeastlike fungi in tissue

* The mature segmented spores contained within the large sporangium of *Rhinosporidium seeberi*, the cause of rhinosporidiosis, are coated with a mucicarminophilic matrix In animal tissue the cigar-shaped spores of *Sporotrichum schenckii* may also be enveloped in a mucicarmine-positive material Because of the obvious morphologic dissimilarities of these two organisms to *C neoformans*, their differentiation should not offer difficulties

TECHNIC.

a Cut paraffin sections of any well fixed tissue at 6 microns thickness and affix to microslides with albumin or gelatin, dry
b Deparaffinize to distilled water
c. Stain for a few seconds in Weigert's hematoxylin (equal parts of freshly mixed solutions "A" and "B").

Solution A
One per cent hematoxylin in 95 per cent ethyl alcohol

Solution B

Ferric chloride, 29 per cent aqueous	4 ml
Distilled water	95 ml
Hydrochloric acid, concentrated	1 ml

d Wash in tap water
e Stain in Metanil yellow solution for 1 minute

Metanil yellow	0 25 Gm
Distilled water	100 0 ml
Glacial acetic acid	0 25 ml

f Rinse in distilled water
g Place in diluted mucicarmine solution for 30 to 60 minutes, compare microscopically with control slide.

Carmine	1 0 Gm
Anhydrous aluminum chloride	0 5 Gm
Distilled water	2 0 ml

Mix in a small test tube and heat over a small flame for 2 minutes until liquid becomes dark and syrupy Pour into 100 ml 50 per cent ethyl alcohol and permit it to stand 24 hours Dilute 1 to 4 for use

h Rinse quickly in 95 per cent ethyl alcohol, then in two changes absolute alcohol
i Clear in two changes xylene and mount in Permount

RESULTS Cryptococcus capsule and cell wall, pink to red Mucinous substances, pink to red Nuclei of tissue cells, black Other tissues, yellow

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20 GOMORI'S METHENAMINE-SILVER NITRATE TECHNIC[81] (modified slightly at the Armed Forces Institute of Pathology)

USE. The Gomori stain, a histochemical test for glycogen and mucin and the newest technic to be applied to the histopathologic demonstration of fungi in tissue sections, has the following advantages. it is unparalleled for detecting small numbers of fungi which might be missed in lesions stained by other technics, it is the only fungus stain which satisfactorily demonstrates certain strains of *Mucor,* and it yields the most photogenic preparations for black-and-white photography.

PRINCIPLE The Gomori method is considered to be a specific histochemical test for aldehydes and is not a conventional silver impregnation involving pure reduction technics As with the Gridley method (Appendix formula 17) the first step in the staining process is the liberation of aldehyde groups of the fungal cells by treatment with chromic acid, with their subsequent detection by the reduction of an alkaline methenamine-silver nitrate complex When the sections are placed in an alkaline methenamine-silver nitrate solution, the histochemical reaction which occurs precipitates reduced silver on the fungal elements.

TECHNIC

a Cut well-fixed, paraffin-imbedded tissues at 6 microns thickness and affix to microslides with albumin or gelatin (dry smears may be prepared on albumin-treated slides and fixed in 95 per cent ethyl alcohol)

b Deparaffinize to distilled water

c Oxidize in 5 per cent chromic acid (CrO_3) for 1 hour

d Wash in running tap water for 10 minutes

e Treat in 5 per cent sodium bisulfite ($NaHSO_3$) for 1 minute and wash again in tap water for 5 minutes

f Rinse in 3 changes of distilled water.

g Silver the preparation at 45–50°C in a working solution prepared by adding 25 ml of stock methenamine-silver nitrate to an equal portion of distilled water containing 2 ml of 5 per cent borax, U S P. grade ($Na_2B_4O_7 \cdot 10H_2O$).

Stock methenamine-silver nitrate solution

3 per cent methenamine, U S.P. grade 100 ml.

5 per cent silver nitrate 5 ml

A white precipitate forms which dissolves immediately upon shaking, the clear solution remains stable for months while refrigerated

Staining of fungi and mucin begins within 25 to 30 minutes but satisfactory results are not obtained until after 1 hour or more has elapsed The staining reaction should be followed closely after 1 hour

h Rinse in distilled water 3 times

i Tone in 0 1 per cent gold chloride ($AuCl_3 \cdot HCl \cdot 3H_2O$) solution for 5 minutes to bleach the background

j Rinse in distilled water

k Treat with 2 per cent sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) solution for 1 or 2 minutes to remove unreduced silver

l A variety of counterstains including hematoxylin-eosin, light green, metanil yellow, and aqueous safranine may be used to good advantage but for black-and-white photography the preparations should not be counterstained

m Dehydrate, clear, and mount in the usual manner

RESULTS As a result of their being coated with reduced silver, fungal spores and hyphae, including *Mucor*, are sharply outlined in black against a pale background Glycogen, mucin, and elastin are also demonstrated by this technic

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Index

Note Words in bold face indicate reference to subjects of major interest
Page numbers in ***bold face italic*** refer to illustrations.

A

Acid-fast staining for ascospores, 103
Acid mucopolysaccharide stain for *C neoformans*, ***116***, 117
Acneform lesions of skin, ***24***, ***26***, ***29***, ***64***
Acriflavine in cryptococcosis, 126
Actidione
absence in spinal fluid, 143
administered with hyaluronidase, 142, 146
administered with polymyxin, 142, 146
administered with streptodornase, 142, 146
assay methods, 140
blood levels in patients, 142
chemical structure, 140
effect of whole blood, 141
effect on *C neoformans*, 136, 138, 139
efficacy in cryptococcal meningitis, 142
for cryptococcal meningitis, 139, 141, 142, 143
for mouse cryptococcosis, 141
fungicidal activity, 139
inactivation by alkali, 140
mode of administration, 142
mouse LD_{50}, 138, 140
mouse toxicity, 138, 140, 141
serum level in guinea pig, 141
source, 139
synergistic action with polymyxin, 142
tissue concentration, 143
toxicity in animals, 138, 139, 140
toxicity in humans, 142
treatment in cryptococcosis, 75, 125, 126, 127, 136, 138, 139, 140, 141, 142, 143, 146
Actinomycin, 138
mouse toxicity, 138
Adrenal cryptococcosis, ***64***, ***86***, 87
Adrenal insufficiency, ***64***
Age distribution of cryptococcosis, 7
Agglutinin-absorption, 47
Agglutinin titers in cryptococcosis, 47, 48
Alcohol, intravenous in cryptococcosis, 126
Alkali digestion of *C neoformans*, 100
Alkali inactivation of Actidione, 140
Alkalinization therapy in cryptococcosis, 126, 127
with hyperthermia, 127
Allergenicity of fungi, 47
Allicin, 144
chemical formula, 144
effect on *C neoformans*, 136, 145
mouse LD_{50}, 145
source, 144
toxicity, 145
Allium sativum, 144
Aminostilbamidine, effect on *C neoformans*, 132
Amputation in cryptococcosis, 29, 125
Anascosporogenous yeasts, 2
Anemia, in cryptococcosis, 91
Aneurysm, 35
Animal cryptococcosis, 38
tissue response, 40, ***40***, ***41***
Anisotropism, 116
Antibiotics against fungi
derived from actinomycetes, 138, 147
derived from bacteria, 136, 137
derived from fungi, 137
Antibiotic therapy in cryptococcosis, 126, 136
Antibiotic XG, 136, 137
mouse LD_{50}, 136
Antibody titers in cryptococcosis, 47, 48
Anticryptococcal drug, ideal properties, 125
Anticryptococcal plant extracts (see Plant extracts effective against *C neoformans*)
Antifungal antibiotics
derived from actinomycetes, 138, 147
Actidione, 138, 139
actinomycin, 138
ascosin, 138, 139, 147
C-135, 138
C-381, 138
candicidin, 138, 139, 147
candidin, 138, 147
fradicin, 138
fungichromatin, 148
fungichromin, 138, 147
fungicidin, 138

(*Antifungal antibiotics, derived from actinomycetes—cont'd*)
heptaenes, 147
hexaenes, 147
musarin, 138
mycoticin, 138, 148
nystatin, 138, 139, 148
pentaene, 147
polyene chromophore group, 147
rimocidin, 138, 141, 147
similarity to each other, 147
streptomycin, 138
streptothricin, 138
tetraene, 147
thiolutin, 138
derived from bacteria, 136, 137
Antibiotic XG, 136, 137
bacillomycin, 136, 137
circulin, 136, 137
eumycin, 136, 137
fluvomycin, 136, 137
fungistatin, 136, 137
hemi-pyocyanine, 136, 137
mycosubtilin, 136, 137
polymyxin, 136, 137
prodigiosin, 136, 137
pyocyanine, 136, 137
violacein, 136, 137
derived from fungi, 137
citrinin, 137
clavicin, 137
gliotoxin, 134, 137
pleurotin, 137
trichothecin, 137
ustilagic acid, 137
viridin, 137
derived from higher plants, 143
Coptis chinensis extract, 144
effect on *C neoformans*, 144
garlic clove extract, 144
effect on bacteria, 145
effect on *C neoformans*, 145
LD_{50} for mice, 145
protoanemonin, 143
chemical formula, 143
chemical nature, 143
effect on *C neoformans*, 143
for cryptococcal meningitis, 143
for osseous cryptococcosis, 143
mouse toxicity, 143
source, 143
toxicity in humans, 144
tomato extract, 144
effect on *C neoformans*, 144
Western Red Cedar extract, 144
effect on *C neoformans*, 144
wheat flour extract, 144
effect on *Saccharomyces cerevisiae*, 144
Antigenic structure of *C neoformans*, 47
Antihistaminic compounds, 135
tissue levels, 135
Antimonials in cryptococcosis, 129
activity of Ro-2-3094, 129
effect on *C. neoformans*, 129
failure in meningitis, 129
Antimony and potassium tartrate, 126, 129
Antimony compounds, 126, 129
Antimony potassium tartrate, 135
Antistine hydrochloride
effect on *C neoformans*, 135
Arsenicals in cryptococcosis, 126
Artefacts in sputum, 96, *97*
Artefacts in tissues resembling cryptococci, 61, 117, 118
Asbestos bodies in sputum, *97*
Ascosin, 139
for mouse histoplasmosis, 139
heptaene, 147
human toxicity, 139
mouse LD_{50}, 138
mouse toxicity, 141
Ascospore formation by *C neoformans*, 2, 96, 103, 104, 147
Ascospore stain, 103
Aspergillic acid, effect on *C neoformans*, 136
Aspergillus clavatus, 137
Aspiration biopsy, 9, 34
Asporogenous yeasts, classification, 109
Assimilation of carbon by yeasts, medium, 155
Assimilation of KNO_3 by yeasts, medium, 156
Assimilation of nitrogen by yeasts, medium, 156
Atabrine in cryptococcosis, 126, 135
Atalectasis, *14*
Atelosaccharomyces breweri, 111
Atelosaccharomyces busse-buschki, 111
Atelosaccharomyces hominis, 111
Aureomycin in cryptococcosis, 126, 136
effect on *C neoformans*, 136
Autogenous vaccine in cryptococcosis, 126, 145, 146
Auxanographic agar for yeasts, 155, 156
Avirulent cryptococci, 6
Azosulfamide in cryptococcosis, 126

B

Bacillomycin
effect on *C neoformans*, 136, 137
mouse LD_{50}, 136
Bacillus circulans, 136
Bacillus polymyxa, 136
Bacillus subtilis, 136, 137
antifungal derivatives, 137

Bacitracin
  effect on *C neoformans*, 136
Basal ganglia cryptococcal lesions, *64*, *76*
Bauer stain for *C. neoformans*, *116*, 117
β-diethylamino ethyl fencholate,
  as antifungal agent, 134
  compared with gliotoxin, 134
  effect on *C neoformans*, 134
  LD_{50} for mice, 134
Biacetyl, effect on *C neoformans*, 150
Biochemical properties of *C. neoformans*, 105
Biopsy of skin, hazard, 99
Blastomyces dermatitidis
  effect of compound P-594, 134
  effect of fungichromin, 148
  effect of propamidine, 130, 132
  effect of stilbamidine, 132
  effect of 2-hydroxystilbamidine, 131, 132
  hematoxylin-eosin stain, 116
Blastomyces lithogenes, 110
Blastomyces neoformans, 110
Blindness, caused by *C. neoformans*, 34
Blood culture, in cryptococcosis, 92, 151
  diagnostic potentialities, 34
Blood levels
  of Actidione, 149
  of antifungal substances with *Candida tropicalis*, 140
  of antifungal substances in guinea pig, 140
    Actidione, 141
    ascosin, 141
    candicidin, 141
    fungicidin, 141
    nystatin, 141
    prodigiosin, 141
    rimocidin, 141
    stilbamidine, 141
  of nystatin, 149
Bone marrow cryptococcal involvement, *90*
  culture, 92, 93
Botanical classification of *C neoformans*, 2, 6, 108, 109
Bovine cryptococcosis, 38, 40, 41, 42, *42*, *43*, *44*, 45
Bovine mastitis, *42*, *43*
Bovine meningoencephalitis due to *C neoformans*, 42
Bovine pulmonary cryptococcosis, *44*
Brain and meninges (see Cryptococcosis of CNS)
Brain-heart infusion blood agar, *100*, *101*, 102, 153
Brettanomyces genus, 108, 109
Bromothiazone, effect on *C neoformans*, 134
Bronchopneumonia due to *C neoformans*, 14
Bronchoscopic aspiration, 9
Bronchoscopic biopsy, 9
Brown and Brenn Gram stain, 161
Bullera, 108
Buttercup extract, 143

C

Calcium gluconate in cryptococcosis, 126
Cancers as source of *C. neoformans*, 2
Candicidin, 138, 139, 147
  for mouse cryptococcosis, 139
  mouse LD_{50}, 138, 139
  toxicity, 138, 139, 141
Candida albicans
  effect of compound P-594, 134
  effect of *fungichromin*, 148
  effect of iodides, 128
  effect of parahydroxybenzoic acid, 133
  effect of tomato extract, 144
Candida curvata, 108
Candida genus, 108, 109
Candida guilliermondii, 147
Candida humicola, 108
Candida melibiosi, 147
Candida tropicalis for serum fungicide assay, 140
Candidin, 138, 147
  mouse LD_{50}, 138
  toxicity, 138
Canine cryptococcosis, 38, *41*
Canine meningoencephalitis due to *C neoformans*, *41*
Capric acid, 134
Capsular polysaccharide of *C neoformans*
  absence of hyaluronic acid, 48
  antigenic types, 47
  chemical composition, 48
  chromatographic analysis, 48
  effect of hyaluronidase, 48
  effect of proteins, 50
  protein-polysaccharide complex, 50
  serologic reactivity, 47
  type B separation, 48
Capsular reaction, *49*, *50*, 99
Capsule, of *C neoformans*, 93
  metachromatic properties, 99
  stained in tissue, *116*
Carbon assimilation by *C neoformans*, 105, *106*
  agar, 155
  broth, 155
  technic, 106
Carbon auxanographic agar method, 105
Cardiovascular cryptococcosis, 35, 88, 151
Carrot infusion sporulation agar for yeasts, 157
Caseation necrosis in cryptococcosis, *59*

Cavitation on X-ray, 14, 15, 16, *18*
Cell wall, of *C. neoformans*, 95
Cerebral cryptococcosis (see Cryptococcosis of CNS)
Cerebral granuloma (see Granuloma)
Cetyl dimethyl ethyl ammonium bromide, effect on *C. neoformans*, 150
Cetyl trimethyl ammonium bromide, effect on *C. neoformans*, 150
Cheetah cryptococcosis, 38
Chemical structure of *C. neoformans*, 47
Chemotherapy in cryptococcosis (see Cryptococcosis, therapy)
Chick embryo, susceptibility to *C. neoformans*, 114
Chloromycetin
  effect on *C neoformans*, *136*
  in cryptococcosis, 126, 136
Chlorquinaldol
  chemical formula, 150
  effect on *C neoformans*, 150
  effect on systemic pathogenic fungi, 150
  toxicity to animals, 150
Choriocapillaris invasion, *35*
Chorioretinitis, *84*, *85*
Choroid plexus lesion, *80*
Chromobacterium prodigiosum, 136, 141
Chromobacterium violaceum, 136
Chromogenesis in fungi, agar, 152
Chromatographic analysis, 48
Circulin, 136, 137
  in cryptococcal meningitis, *137*
  in cryptococcosis, 126, 136
  mouse LD_{50}, 136
Citrinin, 137
  mouse LD_{50}, 137
  toxicity, *137*
Civet cryptococcosis, 38
Classification of asporogenous yeasts, 108
Classification of *C neoformans* (see Botanical classification)
Clavicin, *137*
  mouse, LD_{50}, 137
Clinical course of cryptococcal meningitis, 19
Clinical manifestations of cryptococcosis, 12
Coccidioides immitis
  effect of mycoticin, *148*
  hematoxylin-eosin stain, 116
Coccidioidomycosis, treatment with nystatin, 149
Coin lesions, 15
Colloidal copper in cryptococcosis, *126*
Colloidal silver in cryptococcosis, 126
Colony appearance of *C neoformans*, culture media, 101, 102, 103, *104*
Complement fixing antibodies, 48, 151
Complement fixing antigen, 151
Compound P-591, *131*
Compound VR-701, 150
[illegible]
Coptis chinensis extract, *141*
Cortical atrophy, *79*
Cortisone
  effect on cryptococcal-infected rats, 150
  effect on *Hormodendrum*-infected rats, 150
  in treatment of cryptococcosis, *150*
Cranial involvement, 31
Crepin, effect on *C. neoformans*, 136
Criteria for identification *C. neoformans*
  biochemical, 105, 120
  cellular morphology, 93, 120
  colony formation, 99, 120
  histopathologic, 116, 120
  oxygen requirement, 108, 120
  pathogenicity, 111, 120
  serologic, 47, 120
  tolerance to 37°C, 104, 120
Cryptococcaceae family, 108, 109
Cryptococcal bronchopneumonia (see Cryptococcosis of lungs)
Cryptococcal encephalitis, canine (see Cryptococcosis of CNS)
Cryptococcal granuloma (see Granuloma)
Cryptococcal infected milk, *45*
Cryptococcal polysaccharide, 48
  in urine, *50*
Cryptococcales order, 108
Cryptococci
  avirulent, 6
  occurrence in air, 6, 101
  occurrence *in intestinal tract*, *6*
  occurrence in rodents, 6
  occurrence in soil, 6
  occurrence in sputum, 6, 101
  occurrence on skin, 6, 99, 101
  serologic groups, *47*
Cryptococcoideae subfamily, 108, 109
C-135, 138
C-381, 138
Calcification, pulmonary, 16, *67*
  rarity, *118*
Cryptococcus albidus, 109, 110
Cryptococcus breweri, 111
Cryptococcus cerebriloculosus, 111
Cryptococcus costantini, 111
Cryptococcus diffluens, *109*, *110*
Cryptococcus farciminosus, 45
  cause of epizootic lymphangitis, 45
  classification as *Histoplasma farciminosum*, 45

resemblance to *H. capsulatum*, 45
Cryptococcus genus, 109, 109, 110
Cryptococcus glabrata (see *Torulopsis glabrata*)
Cryptococcus hominis, 1, 2, 111
Cryptococcus hondurianus, 111
Cryptococcus hominis var *hondurianus*, 111
Cryptococcus kleini, 111
Cryptococcus laurentii, 109, 110
Cryptococcus lithogenes, 110
Cryptococcus luteolus, 109, 110
Cryptococcus meningitidis, 111
Cryptococcus mucorugosus, 109, 110
Cryptococcus nasalis, 111
Cryptococcosis
absence of endemic areas, 6
age distribution, 7
as cause of aneurysm, 35
as cause of blindness, 31
associated with Hodgkin's disease, 35, 36, *86*
associated with lymphoma and leukemia, 35, 36
associated with sarcoidosis, 37
cause of epithelioid tubercles, 58
cautery, 128
chemotherapy, 128
coexistence with Hodgkin's disease, 71
coexistence with other mycoses, 70
coexistence with tuberculosis, 135
effect of isonicotinic acid hydrazide, 135
discrepancy in clinical and pathological diagnosis, 4
disseminated, 8, *26*, *27*, 31, *64*, *65*, *86*, *87*
causing miliary pulmonary lesions, 14
in autopsied cases, 8
in reticulo-endothelial disease, 8
eosinophilia, 91
epidemiology, 7
etiologic diagnosis
aspiration biopsy, 9
blood culture, 34
bronchoscopic aspiration, 9
dissemination in Hodgkin's disease, 36
liver biopsy, 34
marrow aspiration, 34
urine culture, 34
relation to sarcoidosis, 36
excision, 128
exogenous origin, 7, 45
frequency in females, 7
frequency in males, 7
historical aspects, 1
in animals, 38
in cat, 38
in cheetah, 38
incidence, 3
in civet, 38, 39
in dog, 38, *41*
in ferret, 38
in fox, 38, 39
in guinea pig, 7, 38
in horse, 2, 38
in monkey, 38, 39
in ox, 38
in pig, 38
in rodents, 112, 113, *113*
in United States, 3
in Washington Zoo, 39
laboratory studies, 58
lymphoma, 35
mucosal lesions, 29
myxomatous-like cryptococcal masses, *25*, 54, *59*, *65*, *66*, *67*, 82
of bone and joints, 30
associated with sarcoidosis, 37
differentiation from other mycoses, 34
extension, *28*, *32*, 34
from meninges, 34
from sinuses, 34
from soft tissues, 32
granuloma, *62*, *87*
in first recorded case, 30
incidence, 30
joint lesions, 32
misinterpreted as neoplasm, 88
of bone marrow, *89*, *90*
of cranium, 21, 31
of femur, 32
of humerus, *31*, *32*
of ilium, 31, 32
of ischium, *33*
of pelvis, 31
of radius, *32*
of tibia, *33*
of tibial tubercle, *33*
of phalanx, *25*
of vertebra, 31
osteomyelitis, *28*, *31*, *32*, *33*
rarity of solitary bone lesions, 32
result of hematogenous dissemination, 32
roentgenograms, *28*, *31*, *32*, *33*, *34*, *35*
roentgenographic diagnosis, 34
sites of occurrence, 32
surgical treatment, 31
symptomatology, 30
treatment with protoanemonin, 143
vaccine therapy, 145
of cardiovascular system, 35, *88*, 151
as cure of aneurism, 35
endocarditis, 151
with rheumatic heart disease, 151
of cat, *39*, *40*
of cattle, 38, 40, *42*, *43*

(*Cryptococcosis—cont'd*)
of central nervous system, 2, 3, 17, 71
choroid plexus lesions, ***80***
clinical manifestations, 17, 19
cortical atrophy, ***79***
differential diagnosis, 21
differentiation
benign lymphocytic choriomeningitis, 23
fungal meningitides, 23
metastatic carcinoma, 21
neurosyphilis, 23
tuberculous meningitis, 19, 21, 22
dural granuloma, ***83***
duration, 20
encephalitis, 74
in cocker spaniel, ***41***
encephalomalacia, 80, ***90***
example of chronicity, 19
first antemortem diagnosis, 2
first case treated with Actidione, ***75***
fulminant cases, 20
gross appearance, ***64, 71, 72, 79, 80, 83***
hematogenous spread in brain, ***76, 77***
hydrocephalus, 19, ***21, 79***, 80, ***80***
ocular disorders, 18
in animals, ***40***, 41
in older patients, 18, 23
in white Swiss mice, ***113***
lentiform nucleus, ***76***
meningitis, 2, ***20, 22***, 31, 71, ***71, 80***
cure with sulfadiazine, 130
differential diagnosis, meningitis, 21
fibrovascular proliferation, ***74***
headache, 17
leukopenic effect of sulfonamides, 130
resembling neoplasm, ***22***
treatment (see Cryptococcosis, treatment)
meningoencephalitis in animals, ***40, 41***
meningoencephalitis in humans, 8, 10, 17, 18, 19, ***19***, 32, ***55, 64, 73, 76, 77, 78, 79***
mistaken for bacterial meningitis, 23
neuropsychiatric symptoms, 23
of basal ganglia, ***76***
of brain, ***56***
of gasserian ganglion, 7
of internal capsule, ***76***
of lentiform nucleus, ***76***
of spinal cord granuloma, 124
papilledema, 18, ***19***
paralysis, 20
pathology
in infected mice, 112, ***113, 114***
of cryptococcal meningitis, 71
distention of subarachnoid space, 72
gross appearance of brain and meninges, 71
meningeal reaction, 71, 73
microscopic characteristics, 73
of cryptococcal encephalitis, 74
brain deformity, 82
cyst formation, 79
effect of formalin fixation, 78
embolic lesions, 77
encephalomalacia-like lesions, 80
endarteritis, ***81***, 82
external hydrocephalus, 82
fibrosis and calcification, 82
granulomatous lesions, ***78***, 80
in newborn, 80
internal capsule, ***76***
internal hydrocephalus, 80
mechanism of involvement, 74
miliary granulations, 80
mineral concretions in newborn, 80
perivascular extension, 74
spinal fluid, 91, 92, 96
of space-occupying granuloma, 82
paucity of reaction, 53
physical and neurological abnormalities, 17
soluble antigen in spinal fluid, 48
spinal fluid dynamics, 20
symptomatology in artificially infected mice, 112
tissue susceptibility to *C. neoformans*, 8
treatment (see also Cryptococcosis, treatment)
fever therapy, 104
first case treated, 141
symptomatic treatment, 146
lumbar puncture, 146
of ambulatory patient, 146
of hospitalized patient, 146
opiates, 146
vaccine therapy, 145
routes of administration, 145
with Actidione, 75, 139
clinical failure, 142
highest I V dose, 143
mode of administration, 142
toxic effects, 142
with polymyxin, 137, 142
with circulin, 137
with ethyl vanillate, 133
with hyaluronidase, 142, 146
with polymyxin, 142, 146
with prodigiosin, 137
with protoanemonin, 143
with stilbamidine, 132

with streptodornase, 142, 146
ventricular dilatation, *21*, *79*, 80, *80*
of eye
blindness by *C neoformans*, 34
difficulty in diagnosis, *85*, 87
enucleation, *64*, *66*, 125
in human, *19*, *35*, *64*, *85*
in rabbit, 114, *115*
keratitis, 35
of eyelids, 35
of optic nerve, *20*, 34
of orbit, 35
of retina, 34, *64*
of uveal tract, 34, *35*
of vitreous, 34
of lungs, 8, 9, *9*, 11, 12, 13, 15, 16, *16*, 46, *61*, 64, 65, *66*, 68, 69, 70, 71
as cause of death, 9, 12
aspiration biopsy, 9
associated with nervous system disease, 9
atalectasis, *14*
bilateral disease, *16*
bronchopneumonia, 14
bronchoscopic aspiration, 9
bronchoscopic biopsy, 9
caseation necrosis, 59
cavitation, *18*
coexistence with Hodgkin's disease, 71
coexistence with other mycoses, 70
coexistence with tuberculosis, 70
complication with non-mycotic pneumonitis, 70
diagnosis by sputum culture, 16
diagnosis by thoracentesis, 12
differential diagnosis, 15
distinguished from tuberculoma, 68
extension to chest wall and mediastinum, 69
fibrocaseous lesions, *63*, 68
gelatinous miliary tubercles, 15
granuloma, *9*, *10*, *18*, *53*, *54*, *59*, *60*, *63*, *69*
granulomatous miliary tubercles, 15
hemoptysis, 11, 12
importance of case follow up, 11
in absence of nervous system disease, 9
in Hodgkin's disease, *86*, *92*
invasion of pleura, 69
involvement of chest wall, 13
in white Swiss mice, *113*
localized lesions, 122
mediastinitis, 13
of bovine lung, *44*
pathology, 64
appearance of pulmonary masses, 64
compared with coccidioidomycosis and histoplasmosis, 68
diagnosis by frozen section, 65
gross examination, 64
infrequency of cavitation, 68
location of pulmonary masses, 64
microscopic appearance, *67*
paucity of tissue necrosis, 68
rarity of pleuritis, 68
resemblance to myxomata, 65, *66*
subpleural granuloma, *9*, *61*, 68
peribronchial infiltration, 14, *15*
physical signs, 12
pleural effusion, *11*
pleural granuloma, 11
pleural reaction, *10*
pleural thickening, *14*
pleuritis, 69
pneumonectomy, 122, 123
pneumonitis, *57*
portal of entry, 6, 7, 8
pulmonary calcification, *63*
pulmonary consolidation, *14*, *18*, *66*
pulmonary granuloma, *9*, *10*, *18*, *53*, *54*, *59*, *60*, *63*, *69*
pulmonary resection, 122
rarely pure respiratory disease, 69
rarity of fistula formation, 69
regression of large lesions, 9
remission of infiltration, 123
resemblance to sarcoidosis, *17*, *57*, *60*, 68
resemblance to tuberculosis, *16*
reticulum framework of lung, *70*
roentgen appearance, *10*, *11*, *12*, *13*, *14*, *15*, *16*, *17*, *18*
roentgen diagnosis, *10*, *11*, *12*, 13, *14*, *16*, 16, *17*, *18*
sarcoid-like pattern, *57*, *69*
spontaneous remission, 123, 125
subpleural granuloma, 8, *9*, 11, *53*, *60*, *61*, *64*, 68
surgical cure, 11
surgical treatment, 9, 121
symptomatology, 11
thoracentesis, 12
with Hodgkin's disease, *86*, *92*
with lymphocytic leukemia, *65*
of newborn
pathology, 80
similarity to toxoplasmosis, 8

(*Cryptococcosis, of other organs—cont'd*)
genito-urinary tract, 34
kidney, 17, 34, ***86***, ***90***, ***93***
liver, 34, ***86***
lymph nodes, 34
oropharynx, 6
pathology, 56
difficulty in diagnosis, 83
dissemination with lymphoma and leukemia, 88
localization of lesions, 83
of adrenals, ***86***, 87, 89
of eye, 87
of kidney, ***86***, 88
of liver, ***86***, 89
of soft tissues, 82
of spleen, ***87***, 89
organ enlargement with infection, 88
prostate gland, 34
spleen, 34, ***87***
testis, 34, ***89***
vagina, 6
of rabbit, ***115***
of skin and mucous membranes, 8, 24, ***24***, ***26***, ***27***, ***28***, ***62***, ***64***, ***84***
acneform lesions, ***24***, ***26***, 29, ***64***
as manifestation of dissemination, 30
as portal of entry, 7
biopsy of skin, hazard, 99
characteristics of cutaneous lesions, 25, 29
characteristics of mucosal lesions, 29
coincident with injury, 30
extension from osseous lesion, ***28***, 29, ***62***, 82
facial lesions in animals, ***38***, 39
first review, 29
granuloma, ***27***
hypersensitivity, 47, 51
incidence, 29
in first recorded case, 24
in granulocytic leukemia, ***26***, ***64***
manifestations, 29
microscopic appearance, ***84***
pathogenesis, 30
pathology, 29, 82
absence of hyperplasia, 82
absence of sinus tracts, 82
localization of cryptococci, 82
resemblance to myxomata and lipoma, ***25***, 82
ulceration, 83
resemblance to rodent ulcer, ***27***
resemblance to smallpox, ***27***
secondary to osteomyelitis, ***62***
strawberry-like lesions, 25
subcutaneous lesions, 25
treatment of mucosal lesions, 29
pathogenesis, 38, 39
pathology (see Pathology of cryptococcosis)
physical agents, 125, 126
prognosis, 121
relation to Hodgkin's disease, 35, 36, 56
spontaneous, 121, 123, 125
symptoms related to single organ, 34
tissue response, resemblance to sarcoidosis, 36
transmission, 7, 45
treatment, medical, 125
antibiotic, 136
of CNS cryptococcosis, expanding intracranial lesions, 124
of extrapulmonary cryptococcosis, 124
symptomatic treatment, 146
treatment summary, 146
with acriflavine, 126
with Actidione (cycloheximide), ***75***, 125, 126, 127, 136, 138, 139, 140, 141, 142, 143, 146
with alcohol, intravenous, 126
with alkalinization therapy, 126, 127
with antibiotics, 126, 136
with antihistamines, 135
with antimony compounds, 126, 129
with arsenicals, 126
with atabrine, 126, 135
with Aureomycin, 126, 136
with autogenous vaccine, 126, 145, 146
with azosulfamide, 126
with calcium gluconate, 126
with circulin, 126, 136
with chemotherapy, 126, 128
with colloidal copper, 126
with colloidal silver, 126
with cortisone, 150
with Daraprim, 149
with diamidine derivatives, 132
with diamidines, 126, 130, 131, 132, 146
with diazone, 126
with enzymes, 126, 145
with ethyl vanillate, 126, 133
with fever therapy, 125, 126, 127
with Fuadin (Stibophen), 126
with furacin, 126
with gamma globulin, 51, 126, 127, 145
with gantrisin, 126
with gentian violet, 126
with gold sodium thiosulfate, 126
with heat cabinet, 126
with heavy metals, 126
with hexamethylenamine, 126
with hexamine, 126

with hyaluronidase (Wydase, Alidase), 126, 127, 142, 145, 146
with immune rabbit serum, 126
with iodides, 126, 127, 128, 145, 146
with lipiodal, 126
with M & B 693, 126
with methylene blue, 126
with nystatin, 126, 138, 139, 141, 148
with oxophenarsine hydrochloride, 126
with para-amino-salicylic acid, 126
with penicillin, 126, 127, 136
with physical agents, 125, 126
with polymyxin, 126, 127, 136, 142, 145, 146
with Pregl's iodine solution, 126
with prodigiosin, 126, 136, 137, 141
with promin, 126
with protoanemonin, 126, 136, 143
with pyribenzamine, (Tripelennamine), 126
with sodium bicarbonate, 126, 127
with sodium thiosulfate, 126
with stilbamidine, 126, 128, 130, 132, 140, 141
with streptodornase, 126, 127, 142, 145, 146
with streptomycin, 126, 127, 136, 138
with sulfadiazine, 126, 127, 129, 130, 145
with sulfamerazine, 126, 129
with sulfanilamide, 126, 129, 130
with sulfapyridine, 126, 130
with sulfathiazole, 126, 129
with sulfonamides, 126, 129, 130, 146
with terramycin, 126, 136
with thiamin, 126
with thymol, 126
with tryparsamide, 126
with 2-hydroxystilbamidine, 126, 128, 130, 131, 132, 146
with typhoid-paratyphoid vaccine, 126, 127, 145
with tyrocidin, 126, 136
with ultra violet radiation, 126
with vaccine therapy, 126, 145, 146
with x-ray, 125, 126, 127, 128
treatment, surgical, 20, 21, 121
amputation, 29, 125
danger of incision of granulomas, 122
enucleation, 85
favorable prognosis in, 121
for prevention of metastasis, 121
importance of case follow-up, 122
of extrapulmonary lesions, 124
of localized lesions, 121, 124, 125
of localized pulmonary lesions, 122, 123
of multiple extrapulmonary lesions, 124
of pulmonary lesions, 121
surgical cures, 21
surgical treatment, 20, 21, 121
vaccine therapy, 126, 145, 146
world distribution of cases, 6
Cryptococcus neoformans
antigenic structure, 47
as cause of mycotic meningitis, 3
as cause of sarcoid-like lesions, 56, 57
as etiologic agent of Hodgkin's disease, 35
ascospore formation, 2, 61, 103, 104, 147
associated with leukemia, 4
associated with lymphoma, 4
biochemical properties, 105
botanical classification, 108, 109, 110
capsular polysaccharide, 48
capsule, 49, 93, ***94***, 110
effect of acid, 47
in tissue, 116
staining reaction, 99, 116, ***116***
carbon assimilation, 105, 106, 110
causing endocarditis, 151
causing mastitis, 40
chemical structure, 47
colony appearance
brain-heart infusion blood agar, ***100***, 102, ***104***
Littman liver-spleen glucose blood agar, ***100***, 103, ***104***
Littman oxgall agar, ***100***, 102, ***104***
Sabouraud dextrose agar, ***100***, 101, ***104***
yeast morphology agar, 103
complement-fixing antigen, 151
criteria for identification, 126
culture media, ***100***, 101, ***104***
culture studies, 99
cytology, 93
cytology differentiation from *C. neoformans* var. *innocuous*, 96
demonstration of capsule in tissue, ***116***
destruction by pasteurization, 7
differentiation,
artefacts, 96
C. neoformans var. *innocuous*, 112
hydropic inflammatory cells, ***118***
mineral concretions, ***119***
other dimorphic fungi, 100
other species, 109, 110
red blood cells, 99
Russell bodies, ***115***, ***119***
direct examination of fluids, 96
double budding, 95
effect of fungicides
Actidione, 136, 138, 139, 141, 143
actinomycin, 138

(*Cryptococcus neoformans, effect of fungicides,—cont'd*)
allicin, 136
aminostilbamidine, 132
antimony potassium tartrate, 129, 135
antistine hydrochloride, 135
ascosin, 138, 139
aspergillic acid, 136
atabrine, 135
Aureomycin, 136
bacillomycin, 136, 137
bacitracin, 136
β-diethylamino ethyl fencholate, 134
biacetyl, 150
bromothiazone, 134
C–135, 138
C–381, 138
candicidin, 138, 139
capric acid, 134
cetyl dimethyl ethyl ammonium bromide, 150
cetyl trimethyl ammonium bromide, 150
Chloromycetin, 136
chlorquinaldol, 150
circulin, 136
citrinin, 137
clavicin, 137
compound P-594, 134
copper sulfamerazine, 130
copper sulfathiazole, 130
copper-3 phenyl salicylate, 134
Coptis chinensis extract, 144
cortisone, 150
crepin, 136
Daraprim, 149
diamthazole, 134
dichlorophenarsine, 135
diethylstilbestrol, 135
dimethyl dichlorosuccinate, 150
diphenylpyraline, 135
dithiocarbamyl hydrazine, 134
dithiocyanoacetanilide, 150
8 hydroxyquinoline, 150
eschridine, 150
estradiol, 150
eumycin, 136
fatty acid derivatives, 134
5-amino-7-hydroxy-1-triazole, 135
fluxomycin, 136
4-allylamino-4'-amino-diphenylsulfone, 129
4,4'-dihydroxy-3,3'di(diethylamino-methyl)-5,5'-diallyl-diphenyl *dihydrochloride*, 135
4,2'-diaminophenyl-5'-thiazolylsulfone (Promizole®), 135
fradicin, 138
Fuadin, 129
fungicidin, 138
fungistatin, 136
garlic clove extract, 145
gladiolic acid, 136
gliotoxin, 134, 137
gramicidin, 136
hexachlorophene, 150
hyamine-1622, 135
hyamine 10x, 135
hydroxystilbamidine, 132
increased pH, 127
isonicotinic acid hydrazide, 135
kojic acid, 136
MRD-112, 134
methyl-5-nitro-2-furoate, 150
Mycostatin, 138
mycoticin, 148
myvisone, 134
N-butylmaleimide, 135
neoarsphenamine, 135
neopyrithiamine, 149
nicotinamide, 135
nitrofurazone (Furacin®), 135
nitro furfuryl methyl ether, 134
N¹-p-nitrobenzoyl sulfanilamide, 129
nystatin, 138
p-aminobenzoic acid, 135
p-aminohippuric acid, 135
p-hydroxymethyl benzoate, 133
p-nitrobenzoic acid, 135
parahydroxybenzoic acid esters, 133
pasteurization, 45, 46
pelargonic acid, 134
penicillic acid, 136
penicillin, 136
penicillin G, 136
penicillin K, 136
pentamidine, 132
phenamidine, 132
phenazine di-N-oxide, 135
phenothiazine, 135
pleurotin, 136, 137
polymyxin, 136
polymyxin with Actidione, 142
propamidine, 132, 135
protoanemonin, 136, 143
pseudomethyl acetylacrylate, 150
pyribenzamine, 135
pyrimidine, 135
quinacrine (Atabrine®), 135
quinine, 135
resorcinol, 135
rimocidin, 138
Ro-2-3094, 129

silver mercaptoacetyl sulfathiazole, 129
6-cyano-2-methoxy-9-isopropyl-aminopropylaminoacridine, 135
6-methoxy-8-isopropyl-amino-pentylaminoquinoline, 135
6-methoxy-8-morpholino-propylamino quinoline, 135
6-phenyl-5,6-dihydrouracil, 135
sodium bismuth mercaptoacetyl sulfathiazole, 129
sodium-5-sulfanilamido-naphthalene-1-sulfonate, 129
sodium p,p'diaminodiphenylsulfone-N,N'-didextrose sulfonate (Promin®), 135
sodium sulfanilyl-sulfanilate, 129
sodium-2-sulfanilamido-benzoate, 129
Spanish moss extract, 144
stibophen (Fuadin®), 135
stilbamidine, 132
streptomycin, 136, 138
streptothricin, 138
subtilin, 136
sulfadiazine, 129
sulfamerazine, 129
sulfanilamide, 129
sulfanilamido-acetic acid, 129
sulfathiazole, 129
Synkamin, 150
thiamine, 100, 149
thiocarbamates, 134
thiocarbamyl hydrazine, 134
thiolutin, 138
thiosemicarbazones, 133
thiourea, 134
3 5-dibromosulfanilanilide, 129
3-phenyl hydantoin, 135
tibione, 134
tomato extract, 144
2-(dimethylaminomethyl)-1-naphthol hydrochloride, 135
2-metanilamido-5-chloropyrimidine 135
tyrocidin, 136
tyrothricin, 136
undecylenic acid, 134
ustilagic acid, 137, 138
vanithiazone, 134
viridin, 137
Western red cedar extract, 144
x-rays, 128
effective antibiotics, 136
endotoxin, 48
failure of Gram stain, 96
fermentation reactions, 105
from the horse, 2
gelatin liquefaction, 108
giant colony on
Littman liver-spleen glucose blood agar, *104*
Littman oxgall agar, *104*
Sabouraud dextrose agar, *104*
Gram-stained preparation, 99
growth on culture media, 99, 100
at 37°C, 110
growth on acid-fast culture media, 100
heat resistance, 104
heat sensitivity, 7
human sources of pathogenic strains, 6
identification in tissues, 116
differentiation from H capsulatum, 117
incubation temperature, 100, 101
India ink mount, *49*, *94*, 96
ineffective antibiotics, 136
in gastrointestinal tract, 6, 34, 39, 39, 151
in oropharynx, 6
in pigeon droppings, 38
in saline mount, 94
in vagina, 6
in vitamin deficient animals, 6
isolation from cancers, 2
isolation from milk, 2, 6, 7, 40
isolation from peaches, 1, 2, 6
isolation from pigeon manure, 7
isolation from pigeon nests, 7
isolation from soil, 7
lipoidal granules, *90*, *95*
mated with C neoformans var innocuous, 147
means of reproduction, 93
most valid terminology, 2
need for precise identification, 99
nitrate assimilation, 106, 107, 110
nonmucoid primary colonies, 101, *104*
occurrence in nature, 6
occurrence in intestinal tract, 6
on blood agar, *104*
on skin, 6 99
oxygen requirement, 108
pathogenesis in Hodgkin's disease, 36
pathogenicity and virulence, 110, 111
animals, 111
chick embryo, 114, 116
eye, rabbit, 114, *115*
guinea pig, 114
rabbit, 114
rats, 113
white Swiss mice, 112, *113*
physiological reactions, 105
polysaccharide, *47*, 49
portal of entry, 6, 34, 39, 39, 151
pseudomycelium in culture, *95*, *95*, *98*
pseudomycelium in tissue, *87*, 117
production of starch-like compounds, 107

(*Cryptococcus neoformans*, *effect of fungicides,—cont'd*)
allicin, 136
aminostilbamidine, 132
antimony potassium tartrate, 129, 135
antistine hydrochloride, 135
ascosin, 138, 139
aspergillic acid, 136
atabrine, 135
Aureomycin, 136
bacillomycin, 136, 137
bacitracin, 136
β-diethylamino ethyl fencholate, 134
biacetyl, 150
bromothiazone, 134
C-135, 138
C-381, 138
candicidin, 138, 139
capric acid, 134
cetyl dimethyl ethyl ammonium bromide, 150
cetyl trimethyl ammonium bromide, 150
Chloromycetin, 136
chlorquinaldol, 150
circulin, 136
citrinin, 137
clavicin, 137
compound P-594, 134
copper sulfamerazine, 130
copper sulfathiazole, 130
copper-3-phenyl salicylate, 134
Coptis chinensis extract, 144
cortisone, 150
crepin, 136
Daraprim, 149
diamthazole, 134
dichlorophenarsine, 135
diethylstilbestrol, 135
dimethyl dichlorosuccinate, 150
diphenylpyraline, 135
dithiocarbamyl hydrazine, 134
dithiocyanoacetanilide, 150
8-hydroxyquinoline, 150
eschridine, 150
estradiol, 150
eumycin, 136
fatty acid derivatives, 134
5-amino-7-hydroxy-1-triazole, 135
fluvomycin, 136
4-allylamino-4'-amino-diphenylsulfone, 129
4,4'-dihydroxy-3,3'di(diethylaminomethyl)-5,5'-diallyl-diphenyl dihydrochloride, 135
4,2'-diaminophenyl-5'-thiazolylsulfone (Promizole®), 135
fradicin, 138
Fuadin, 129
fungicidin, 138
fungistatin, 136
garlic clove extract, 145
gladiolic acid, 136
gliotoxin, 134, 137
gramicidin, 136
hexachlorophene, 150
hyamine-1622, 135
hyamine 10x, 135
hydroxystilbamidine, 132
increased pH, 127
isonicotinic acid hydrazide, 135
kojic acid, 136
MRD-112, 134
methyl-5-nitro-2-furoate, 150
Mycostatin, 138
mycoticin, 148
myvisone, 134
N-butylmaleimide, 135
neoarsphenamine, 135
neopyrithiamine, 149
nicotinamide, 135
nitrofurazone (Furacin®), 135
nitro furfuryl methyl ether, 134
N'-p-nitrobenzoyl sulfanilamide, 129
nystatin, 138
p-aminobenzoic acid, 135
p-aminohippuric acid, 135
p-hydroxymethyl benzoate, 133
p-nitrobenzoic acid, 135
parahydroxybenzoic acid esters, 133
pasteurization, 45, 46
pelargonic acid, 134
penicillic acid, 136
penicillin, 136
penicillin G, 136
penicillin K, 136
pentamidine, 132
phenamidine, 132
phenazine di-N-oxide, 135
phenothiazine, 135
pleurotin, 136, 137
polymyxin, 136
polymyxin with Actidione, 142
propamidine, 132, 135
protoanemonin, 136, 143
pseudomethyl acetylacrylate, 150
pyribenzamine, 135
pyrimidine, 135
quinacrine (Atabrine®), 135
quinine, 135
resorcinol, 135
rimocidin, 138
Ro-2-3094, 129

Dichlorophenarsine, 135
Diethylstilbestrol, 135
Differential diagnosis
of cryptococcal meningitis, 21
of pulmonary cryptococcosis, 15
Dilation of ventricles by cryptococcal infection, 21, 79, 80, 80
Dimethyl dichlorosuccinate, effect on *C. neoformans*, 150
Diphenylpyraline, effect on *C. neoformans*, 135
Direct examination for *C. neoformans*, 96
Disseminated cryptococcosis, 26, 27, 31, 64, 65, 66, 87
Dithiocarbamyl hydrazine
effect on *C. neoformans*, 134
failure to protect mice, 134
Dithiocyanoacetamide, effect on *C. neoformans*, 150
Dural granuloma, 83

E

8-hydroxyquinoline, effect on *C. neoformans*, 150
Elastic tissue in sputum, 97
Electrocardiographic abnormalities with hyperthermia, 127
Electrophoretic analysis, in cryptococcal meningitis, 51
Encephalitis in cryptococcosis, 74
Encephalomalacia, 80, 90
Endarteritis in brain, 81, 82
Endogenous theory of infection, 6
Endotoxin in *C. neoformans*, 48
Enucleation in cryptococcosis, 64, 66, 125
Enzyme therapy
in cryptococcosis,
basis for treatment, 145
hyaluronidase, 126, 145
streptodornase, 126, 145, 146
streptokinase, 145
with Actidione and polymyxin, 146
in tuberculous meningitis, 145
Eosinophilia, in cryptococcosis, 91
Epidemiology of cryptococcosis, 7
Epithelioid tubercle formation, 17
Epizootic lymphangitis, 45
Equine cryptococcosis, 2, 38
Eschtidine, effect on *C. neoformans*, 150
Estradiol, effect on cryptococcal-infected mice, 150
Ethyl vanillate,
blood levels, 133
chemical nature, 133
effect on *H. capsulatum*, 133
effect on mucous membranes, 133
in cryptococcosis, 126, 133
toxicity, 133
Eumycin, 136, 137
European blastomycosis, 1, 2
Excisional therapy for localized lesions, 8
Exogenous origin of cryptococcosis, 7, 43
Extracellular starch detection, medium, 157
Eye involvement, 31, 35, 64

F

Facial lesions in animals, 38, 39
Fat cells in sputum, 97
Fatty acids
effect on *C. neoformans*, 134
effect on nystatin, 139
Feline cryptococcosis, 38, 39, 40
meningoencephalitis, 40
Femur involvement, 32
Fermentation reactions of *C. neoformans*, 105
Fever therapy in cryptococcosis, 125, 126, 127
Fibrovascular proliferation in meninges, 74
5-amino-7-hydroxy-1-triazole, 135
5,7-dichloro-8-hydroxyquinaldine, 150
Flavomycin, 136, 137
mouse LD_{50}, 136
Formalin fixation, 78
4-allylamino-4'-amino-diphenyl-sulfone, 129
4(β-β-hydroxyethylamino ethyl carbamyl) benzene stibonic acid, (Ro-2-3094), 129
4,4'-dihydroxy-3,3'di (diethylaminomethyl)-5,5'-diallyl-diphenyl dihydrochloride, 135
4-2'-diaminophenyl-5'-thiazolylsulfone, (Promizole®), 135
Fox cryptococcosis, 38
Fradicin, 138
mouse LD_{50}, 138
toxicity, 138
Frozen section diagnosis, 65
Fuadin (Stibophen) in cryptococcosis, 126
Fungichromatin, 148
Fungichromin, 138, 147
chemical characteristics, 147
effect on Blastomyces dermatitidis, 148
effect on *Candida albicans*, 148
from *Streptomyces cellulosae*, 147
inhibitory action, 148
polyene reaction, 147
source, 147
toxicity to mouse, 148
Fungicidin, (see Nystatin)
Fungi imperfecti class, 108
Fungistatin, 136, 137
mouse LD_{50}, 136
Furacin in cryptococcosis, 126

Cryptococcus neoformans, pathogenicity and virulence—cont'd)
reaction in heterologous serum, ***49***
reaction in homologous serum, ***49***
recovery from milk, 38
relation to pigeons, 7
resemblance to artefacts, ***97***
resistance by rabbit, 114
resistance to sulfonamides, 129
reproduction in tissues, 117
sectoring, ***104***
serologic grouping, 47, ***49***, 50
cross reactions, 50
silver nitrate stain, 161
similarity to *Lipomyces starkeyi*, 147
size, 93
source of infection, 6
spinal fluid culture, 96, 99
staining reactions
acid mucopolysaccharide stain, ***116***, 117
Bauer stain, ***116***, 117
Gomori stain, 117
Gram's stain, 96, ***98***, 161
Gridley reticulum stain, ***116***
Gridley stain, ***116***, 117, 159
hematoxylin and eosin, ***116***, 116
Hotchkiss-McManus stain, ***116***, 117, 158
mucicarmine stain, ***116***, 117, 160
periodic acid-Schiff stain, ***116***, 117, 158
toluidine blue, 99, ***116***
starch production, 110
streak plate, on
brain-heart infusion blood agar, ***100***
Littman liver-spleen glucose blood agar, ***100***, ***104***
Littman oxgall agar, ***100***
Sabouraud dextrose agar, ***100***
susceptibility to heat, 46
survival of alkali digestion, 100
Synonymy, 3, 110
synthesis of thiamine-pyrimidine, 149
temperature tolerance, 104
under polarized light, 116
urease formation, 108
virulence for calf, 40
Cryptococcus neoformans var *innocuous*, 99, 101, 109, 110, 112
ascospore production, 103
chemical composition, 48
cytology, 96
culture studies, 99
at 20°C
Littman medium, ***100***, 101, 102, ***104***
Littman oxgall agar, ***100***, 101, 102, ***104***
Mycological agar, 101
Mycophil agar, 101
Sabouraud dextrose agar, 101, ***104***
at 37°C
brain-heart infusion blood agar, ***100***, 101, 102
Littman liver-spleen glucose blood agar, ***100***, 101, 103, ***104***
contrasted with staining methods, 150
differentiation from *C. neoformans*, 101, 112
differentiation from other species, 110
giant colony on Sabouraud dextrose agar, ***104***
mated with *C neoformans*, 147
nitrate assimilation, 106
occurrence in air, 6
occurrence in sputum, 6
on yeast morphology agar, ***101***, ***103***
serologic relationship to *C. neoformans*, 99
similarity to *C. albidus*, 110
similarity to *C. diffluens*, 110
similarity to *C. luteolus*, 110
temperature tolerance, 104
urease formation, 108
Cryptococcus neoformans var. *uniguttulatus*, 101
Cryptococcus plimmeri, 111
Cryptococcus psicrophylicus, 111
Culture maintenance, 101
Cutaneous cryptococcosis (see Cryptococcosis of skin)
Cycloheximide (see Actidione)
Cyst formation, 2, 79
Cysteine, effect on nystatin, 139
Cytology of *C neoformans*, 93
Cytoplasm of C neoformans, *96*

D

Dalmau preparations, 154
Daraprim, 149
blood levels, 149
dosage, 149
effect on *C neoformans*, 149
in treatment of cryptococcosis, 149
Deaths, due to mycoses, 3
Debaryomyces genus, 2
Debaryomyces hominis, 2, 111
Debaryomyces neoformans, 110
Dentate nucleus cryptococcal lesions, ***76***
Desensitization with cryptococcal antigen, 145
Deuteromycetes, 108
Diamidines
in cryptococcosis, ***126***, ***130***, ***131***, ***132***, ***146***
in North American blastomycosis, 130
Diamthazole
effect on *C neoformans*, 134
mouse LD_{50}, 134
Diazone in cryptococcosis, 126

in infected mice, 112, *113*
Hydropic inflammatory cells, differentiated
from cryptococci, *118*
Hypersensitivity in mycotic disease, 47, 51
[illegible] 125, 126, 127
[illegible]
128
[illegible] 128
[illegible]
effect in patients, [illegible]
potentiation of Actidione, 125
with heat cabinet, 127
with typhoid-paratyphoid vaccine, 127

I

Ideal properties of anticryptococcal drug, 125
Ileum involvement by *C. neoformans*, 31, 32
Immune rabbit serum in cryptococcosis, 126
Immunologic agents in cryptococcosis, 126
Immunology in cryptococcosis, 47, 151
agglutinin titers, 47, 48
allergenicity of fungi, 47
antibody titers, 47, 48
complement-fixing antigen, 151
precipitin reaction, 47
preparation of immune serum, 47
skin hypersensitivity, 47, 51
true antigenicity, 47
with thinly encapsulated strains, 47
Incidence of cryptococcosis, 3, 4
effect of geographical location, 7
effect of occupation, 7
influence of race, 7
pulmonary form, 4
India ink examination of *C. neoformans*, *49*, *94*, *96*
Inflammation in cryptococcosis, *90*
Internal capsule cryptococcal lesion, *76*
Internal hydrocephalus caused by *C. neoformans*, 19, *21*, *79*, *80*, 80
Intracerebral injection technic in animals, 112, *113*
Intranasal inoculation
in the guinea pig, 7
in mice, 7
Iodides in cryptococcosis, 126, 127, 128, 145, 146
causing liberation of fungus products, 129
deleterious effect in patients, 129
effect on granulomatous tissue, 128
effect on growth of *C. albicans*, 128
failure in CNS disease, 129
Isonicotinic acid hydrazide
effect on *C. neoformans*, 135
effect on co-existing infection, 135

K

[illegible] 130
[illegible]
Kloeckera genus, [illegible]
Kojic acid, effect on *C. neoformans*, 136

L

Laboratory studies, in cryptococcosis, 58
absence of eosinophilia, 91
anemia, 91
changes in sedimentation rate, 91
leukocytosis, 91
spinal fluid examination, 91, *94*, 96
sputum examination, 91
LD_{50} in mouse,
Actidione, 138, 140
allicin, 145
Antibiotic XG, 136
ascosin, 138
bacillomycin, 136
β-diethylamino ethyl fencholate, 134
candicidin, 138, 139
candidin, 138
chlorquinaldol, 150
circulin, 136
citrinin, 137
clavicin, 137
diamthazole, 134
5,7-dichloro-8-hydroxyquinaldine, 150
fluvomycin, 136
fradicin, 138
fungicidin, 138
fungistatin, 136
MRD-112, 134
Mycostatin, 138
mycoticin, 138
nystatin, 138
polymyxin, 138
rimocidin, 138
Ro-2-3094, 129
stilbamidine, 141
streptomycin, 138
streptothricin, 138
thiolutin, 138
Lentiform nucleus cryptococcal lesion, *76*
Leptomeningitis, due to cryptococcus, 2
Leukemia and cryptococcosis, 35, *65*
Leukocytosis, in cryptococcosis, 91
Lipiodal in cryptococcosis, 126
Lipoidal granules in *C. neoformans*, *90*, *95*, *96*
Lipomyces lipoferus, 147

G

Gamma globulin
  administration in cryptococcosis, 51, 126, 127
  in infection, 51
  level in cryptococcal meningitis, 51
Gantrisin in cryptococcosis, 126
Garlic clove extract, 144
Gastro-intestinal cryptococcosis, cat, *39*
Gelatin liquefaction by *C neoformans*, 108
Generalized cryptococcosis, treated with Daraprim, 149
Gentian violet in cryptococcosis, 126
Gitter cells in cryptococcal encephalomalacia, ***90***
Gladiolic acid, effect on *C neoformans*, 136
Gliotoxin
  effect on *C neoformans*, 134
  mouse toxicity, 137
Glucuronic acid, in cryptococcal polysaccharide, 48
Gold sodium thiosulfate in cryptococcosis, 126
Gomori stain, 151
Gram stain of *C neoformans*, 96, ***98***, 117, 161
  in tissue, 161
Gramicidin, effect on *C neoformans*, 136
Granuloma
  bovine lymph node, ***43***
  brain, 82
  cerebrum, ***23***, 27
  CNS, 23
  danger of incision, 122
  dura, ***83***
  palate, tiger, ***38***
  pleura, 11
  pulmonary, ***9***, ***10***, ***18***, ***53***, ***54***, ***59***, ***60***, ***69***
  skin, ***27***
    resemblance to rodent ulcer, ***27***
  spinal cord, 124
  subcutaneous tissue, ***25***
    resemblance to myxoma, ***25***
  subpleural, ***9***, 11, ***53***, ***61***, ***64***
Granulomatous leptomeningitis, ***74***
Granulomatous miliary tubercles, 15
Granulomatous parenchymatous lesions in CNS, ***78***
Granulomatous thickening of choroid plexus, ***80***
Gridley fungus stain for *C neoformans*, ***116***, 117, 159
Gridley reticulum stain for *C neoformans*, ***116***
Guinea pig cryptococcosis, 7, 38, 114

H

Haploid mating types, 147
46
Heavy metals in cryptococcosis, 126
Hefe, 1
Hematogenous dissemination, 32
  to brain, ***76***, ***77***
Hematoxylin-eosin stain for *C. neoformans*, 116, ***116***
Hemi-pyocyanine, 136, 137
Hemoptysis in pulmonary cryptococcosis, 11, 12
Henrici's vegetable juice sporulation agar for yeasts, 157
Hepatic cryptococcosis, ***86***
Heptaenes, 147
Hexachlorophene, effect on *C neoformans*, 150
Hexaenes, 147
  infection, 116
Histoplasma capsulatum
  differentiation from *C. neoformans*, 84
  effect of ethyl vanillate, 133
  effect of 2-hydroxystilbamidine, 103, 131
  resemblance to *C farcinimosus*, 45
  resemblance to *T glabrata*, 78
Histoplasma farcinimosum, 45
Histoplasmosis
  effect of ascosin, 139
  simulated by *T glabrata*, 111
Historical aspects of cryptococcosis, 1
Hodgkin's disease, associated with cryptococcosis, 35, 36, ***86***
Horse cryptococcosis (see Equine cryptococcosis)
Hotchkiss-McManus stain, ***116***, 117, 158
Huang-Lien (see *Coptis chinensis*)
Human meningoencephalitis due to *C neoformans*, 8, 10, 17, 18, ***19***, 32, ***55***, ***64***, ***73***, ***76***, ***77***, ***78***, ***79***
Humerus involvement by *C neoformans*, ***31***, ***32***
Hyaluronidase (Wydase, Alidase), 126, 127 142, 145, 146
  effect on *C neoformans*, 48
  in cryptococcal meningitis, 142, 146
Hyamine-1622, 135
Hyamine 10x, 135
Hydrocephalus
  due to *C neoformans*, 19, ***21***, ***79***, ***80***

in infected mice, 112, *113*
Hydropic inflammatory cells, differentiated from cryptococci, *118*
Hypersensitivity in mycotic disease, 47, 51
Hyperthermia in cryptococcosis, 125, 126, 127
beneficial effect in chick embryos, 125
causing electrocardiographic abnormality, 128
causing uncontrollable temperature rise, 128
combined with alkalinization, 127
detrimental aspects to patient, 128
effect in patients, 126
potentiation of Actidione, 125
with heat cabinet, 127
with typhoid-paratyphoid vaccine, 127

I

Ideal properties of anticryptococcal drug, 125
Ileum involvement by *C neoformans*, 31, 32
Immune rabbit serum in cryptococcosis, 126
Immunologic agents in cryptococcosis, 126
Immunology in cryptococcosis, 47, 151
agglutinin titers, 47, 48
allergenicity of fungi, 47
antibody titers, 47, 48
complement-fixing antigen, 151
precipitin reaction, 47
preparation of immune serum, 47
skin hypersensitivity, 47, 51
true antigenicity, 47
with thinly encapsulated strains, 47
Incidence of cryptococcosis, 3, 4
effect of geographical location, 7
effect of occupation, 7
influence of race, 7
pulmonary form, 4
India ink examination of *C neoformans*, 49, *94*, *96*
Inflammation in cryptococcosis, *90*
Internal capsule cryptococcal lesion, *76*
Internal hydrocephalus caused by *C neoformans*, 19, *21*, *79*, *80*, 80
Intracerebral injection technic in animals, *112*, *113*
Intranasal inoculation
in the guinea pig, 7
in mice, 7
Iodides in cryptococcosis, 126, 127, 128, 145, 146
causing liberation of fungus products, 129
deleterious effect in patients, 129
effect on granulomatous tissue, 128
effect on growth of *C albicans*, 128
failure in CNS disease, 129
Isonicotinic acid hydrazide
effect on *C neoformans*, 135
effect on co-existing infection, 135

K

Kala-azar, treatment with propamidine, 130
Keratitis, cryptococcal, 35
Kligman's modification of Hotchkiss-McManus stain, 158
Kloeckera genus, 108, 109
Kojic acid, effect on *C. neoformans*, 136

L

Laboratory studies, in cryptococcosis, 58
absence of eosinophilia, 91
anemia, 91
changes in sedimentation rate, 91
leukocytosis, 91
spinal fluid examination, 91, *94*, 96
sputum examination, 91
LD₅₀ in mouse,
Actidione, 138, 140
allicin, 145
Antibiotic XG, 136
ascosin, 138
bacillomycin, 136
β-diethylamino ethyl fencholate, 134
candicidin, 138, 139
candidin, 138
chlorquinaldol, 150
circulin, 136
citrinin, 137
clavicin, 137
diamthazole, 134
5,7-dichloro-8-hydroxyquinaldine, 150
fluvomycin, 136
fradicin, 138
fungicidin, 138
fungistatin, 136
MRD-112, 134
Mycostatin, 138
mycoticin, 138
nystatin, 138
polymyxin, 136
rimocidin 138
Ro-2-3094, 129
stilbamidine, 141
streptomycin, 138
streptothricin, 138
thiolutin, 138
Lentiform nucleus cryptococcal lesion *76*
Leptomeningitis, due to cryptococcus, 2
Leukemia and cryptococcosis, 35, 65
Leukocytosis, in cryptococcosis, 91
Lipiodal in cryptococcosis, 126
Lipoidal granules in *C neoformans*, *90*, *95*, 96
Lipomyces lipoferus, 147

Lipomyces starkeyi, 147
absence of growth at 37°C, 147
growth at 20°C, 147
pathogenicity, 147
similarity to *C. neoformans*, 147
Littman liver-spleen glucose blood agar, ***100***, 101, 103, ***104***, 154
giant colony, ***104***
streak plate, ***100***, ***104***
Littman Medium, ***100***, 101, 102, ***104***, 153
Littman oxgall agar, ***100***, 101, 102, ***104***, 153
giant colony, ***104***
streak plate, ***100***
Liver biopsy, ***34***
Localized cryptococcal infection, 8
diagnostic difficulties, 117
in skin, 82
Lumbar puncture in symptomatic treatment, 146
Lycopersicin, 144
Lycopersicon pimpinelliform extract, 144
effect on *Candida albicans*, 144
effect on *C neoformans*, 144
effect on dermatophytes, 144
effect on wilt-fungi, 144
Lymph node cryptococcal lesion, bovine, ***43***
Lymphangitis, epizootic, 45
Lymphoma, associated with cryptococcosis, 35

M

M & B 693 in cryptococcosis, 126
Macrophages, effect of cryptococci, ***56***
Malachite green stain for ascospores, 103
Malt extract agar, 101
Mannose, in cryptococcal polysaccharide, 48
Maple pollen in sputum, ***97***
Maryland enzootic in cattle, 40
Mastitis in dairy cattle, 7, 40, ***42***, ***43***
caused by contaminated milking machines, 40
clinical symptomatology, 41, ***42***, 42
control measures, 42
duct obstruction, 42
economic loss, 42
effect on output, 42
lymph node involvement, 40, ***43***
pulmonary involvement, 40, ***44***
source of infection, 42
Mayer's mucicarmine stain, 160
Mediastinitis, pulmonary cryptococcosis, 13, 37
Medical treatment of cryptococcosis, 126
Meningeal reaction in cryptococcosis, 71
Meninges, tissue susceptibility, 8
Meningitis due to *C neoformans*, ***64***, ***71***, ***72***, ***73***, ***75***, ***81***
granulomatous, ***75***, ***81***
Meningoencephalitis due to *C. neoformans*
in animals, ***40***, ***41***
in cat, ***40***
in cattle, 42
in cocker spaniel, ***41***
in human, 10, 17, 18, 19, ***19***, 32, ***55***, ***64***, ***73***, ***76***, ***77***, ***78***, ***79***
Metastatic involvement, prevention, 8
Methylene blue in cryptococcosis, 126
Methyl-5-nitro-2-furoate, effect on *C. neoformans*, 150
Miliary pulmonary cryptococcosis, 14, ***17***, ***65***
Miliary tubercles, 15, 65
Milk, as source of *C neoformans*, 2, 38, ***45***, 67
Mineral concretions in tissues
differentiated from cryptococci, ***119***
in brain, 80
Monkey cryptococcosis (*see* Simian cryptococcosis)
Morphology agar for yeasts, 154
Mouse
cryptococcosis, ***113***
susceptibility to *C. neoformans*, 112
toxicity of fungicides
Actidione, 138, 140, 141
actinomycin 138
allicin, 145
Antibiotic XG, 136
ascosin, 138, 141
bacillomycin, 136
candicidin, 138, 139, 141
candidin, 138
chlorquinaldol, 150
circulin, 136
citrinin, 137
clavicin, 137
diamthazole, 134
eumycin, 136
5,7-dichloro-8-hydroxyquinaldine, 150
fluvomycin, 136
fradicin, 138
fungicidin, 138, 141
fungistatin, 136
gliotoxin, 137
hemi-pyocyanine, 136
MRD-112, 134
Mycostatin, 138, 141
mycosubtilin, 136
nystatin, 138, 141
pleurotin, 137
polymyxin, 136
prodigiosin, 136
protoanemonin, 143
purothionin, 144
pyocyanine, 136
rimocidin, 138, 141

stilbamidine, 141
streptomycin, 138
streptothricin, 138
thiolutin, 138
ustilagic acid, 137
violacein, 136
Western red cedar extract, 144
wheat flour extract, 141
MRD-112, 134
mouse LD_{50}, 134
Mucicarmine-positive renal casts, 50
Mucicarmine stain for *C. neoformans*, *116*, 117, 160
Mucopolysaccharide excretion by kidney, *90*
Mucopolysaccharide test, 48
Mucor, effect of stain, 151
Mucosal lesions, 29
Mucous membrane as source of entry, 7
infection with *C. neoformans*, 82
Multiple myeloma, treatment
with propamidine, 130
with 2-hydroxystilbamidine, 131
Musarin, 138
Mycological agar, 101, 152
Mycophil agar, 101, 152
for chromogenesis in fungi, 152
Mycostatin (see Nystatin)
Mycosubtilin, 136, 137
Mycoticin, 138, 148
chemical characteristics, 148
effect on *Blastomyces dermatitidis*, 148
effect on *C neoformans*, 148
effect on *Coccidioides immitis*, 148
effect on *Sporotrichum schenckii*, 148
from *Streptomyces ruber*, 148
toxicity to mice, 148
Myelin globules in sputum, *97*
Myocarditis due to *C neoformans*, *88*
Myvisone, effect on *C neoformans*, 134
Myxomatous-like cryptococcal masses, 25, 54, 58, 65, *66*, 67, 82

N

Naphthoquinones
failure to protect mice, 135
for treatment of superficial mycoses, 134
fungistatic properties, 134
Naturally occurring infections, 7
Necrosis in pulmonary tissue, 18
Needle biopsy for diagnosis, 92
N-butylmaleimide, 135
Neoarsphenamine, 135
Neoformans, species term, 1
Neonatal cryptococcosis, 8
pathology, *80*
Neopyrithiamine, 149
Nicotinamide, 135
Nitrofurazone (Furacin®), 135
Nitro furfuryl methyl ether, effect on *C. neoformans*, 134
Nitrogen assimilation
agar, 156
broth technic, 107, 156
by *C neoformans*, 106
by yeasts, 106
Nitrogen auxanographic agar method, 106
North American blastomycosis, treatment
with pentamidine, 132
with phenamidine, 132
with propamidine, 130, 132
with stilbamidine, 130, 132
with 2-hydroxystilbamidine, 131, 132
N'-p-nitrobenzoyl sulfanilamide, 129
Nystatin, 126, 138, 139, 141, 148
beneficial effect, 149
blood levels, 149
chemical characteristics, 148
effect of blood on activity, 148
effect on *C. neoformans*, 138, 139
effect of cysteine, 139
effect of fatty acids, 139
for coccidioidomycosis, 149
for cryptococcal meningitis, 148
for human cryptococcosis, 37, 126, 139, 148
for mouse cryptococcosis, 139
fungicidal action, 139
intravenous administration, 139, 149
in vitro assay, 148
LD_{50} in mouse, 138
oral use in humans, 139, 149
parenteral toxicity, 148
reversal of activity, 139
sclerosing effect on veins, 149
serum level in guinea pig, 141
spinal fluid levels, 148
stability, 148
tetraene, 147
toxicity for animals, 138, 141
toxicity for humans, 139, 148, 149

O

Ocular cryptococcosis
in cerebral cryptococcosis, 18, *19*
in human, *19*, *35*, *64*, *85*
in rabbit, *115*
Optic nerve, invasion by *C neoformans*, *20*, 34
Osseous cryptococcosis (see Cryptococcosis of bone)
Osteomyelitis, cryptococcal, 28, *31*, *32*, *33*
of humerus, *31*, *32*

Lipomyces starkeyi, 147
absence of growth at 37°C, 147
growth at 20°C, 147
pathogenicity, 147
similarity to *C neoformans*, 147
Littman liver-spleen glucose blood agar, ***100***, 101, 103, ***104***, 154
giant colony, ***104***
streak plate, ***100, 104***
Littman Medium, ***100***, 101, 102, ***104***, 153
Littman oxgall agar, ***100***, 101, 102, ***104***, 153
giant colony, ***104***
streak plate, ***100***
Liver biopsy, 34
Localized cryptococcal infection, 8
diagnostic difficulties, 117
in skin, 82
Lumbar puncture in symptomatic treatment, 146
Lycopersicin, 144
Lycopersicon pimpinelliform extract, 144
effect on *Candida albicans*, 144
effect on *C neoformans*, 144
effect on dermatophytes, 144
effect on wilt-fungi, 144
Lymph node cryptococcal lesion, bovine, ***43***
Lymphangitis, epizootic, 45
Lymphoma, associated with cryptococcosis, 35

M

M & B 693 in cryptococcosis, 126
Macrophages, effect of cryptococci, ***56***
Malachite green stain for ascospores, 103
Malt extract agar, 101
Mannose, in cryptococcal polysaccharide, 48
Maple pollen in sputum, ***97***
Maryland enzootic in cattle, 40
Mastitis in dairy cattle, 7, 40, ***42, 43***
caused by contaminated milking machines, 40
clinical symptomatology, 41, ***42***, 42
control measures, 42
duct obstruction, 42
economic loss, 42
effect on output, 42
lymph node involvement, 40, ***43***
pulmonary involvement, 40, ***44***
source of infection, 42
Mayer's mucicarmine stain, 160
Mediastinitis, pulmonary cryptococcosis, 13, 37
Medical treatment of cryptococcosis, 126
Meningeal reaction in cryptococcosis, 71
Meninges, tissue susceptibility, 8
Meningitis due to *C. neoformans*, ***64, 71, 72, 73, 75, 81***
granulomatous, ***75, 81***
Meningoencephalitis due to *C. neoformans*
in animals, ***40, 41***
in cat, ***40***
in cattle, 42
in cocker spaniel, ***41***
in human, 10, 17, 18, 19, ***19***, 32, ***55, 64, 73, 76, 77, 78, 79***
Metastatic involvement, prevention, 8
Methylene blue in cryptococcosis, 126
Methyl-5-nitro-2-furoate, effect on *C neoformans*, 150
Miliary pulmonary cryptococcosis, ***14, 17, 65***
Miliary tubercles, 15, ***65***
Milk, as source of *C neoformans*, 2, 38, ***45***, 67
Mineral concretions in tissues
differentiated from cryptococci, ***119***
in brain, 80
Monkey cryptococcosis (see Simian cryptococcosis)
Morphology agar for yeasts, 154
Mouse
cryptococcosis, ***113***
susceptibility to *C. neoformans*, 112
toxicity of fungicides
Actidione, 138, 140, 141
actinomycin, 138
allicin, 145
Antibiotic XG, 136
ascosin, 138, 141
bacillomycin, 136
candicidin, 138, 139, 141
candidin, 138
chlorquinaldol, 150
circulin, 136
citrinin, 137
clavicin, 137
diamthazole, 134
eumycin, 136
5,7-dichloro-8-hydroxyquinaldine, 150
fluvomycin, 136
fradicin, 138
fungicidin, 138, 141
fungistatin, 136
gliotoxin, 137
hemi-pyocyanine, 136
MRD-112, 134
Mycostatin, 138, 141
mycosubtilin, 136
nystatin, 138, 141
pleurotin, 137
polymyxin, 136
prodigiosin, 136
protoanemonin, 143
purothionin, 144
pyocyanine, 136
rimocidin, 138, 141

stilbamidine, 141
streptomycin, 135
streptothricin, 138
thiolutin, 138
ustilagic acid, 137
violacein, 138
Western red cedar extract, 141
wheat flour extract, 141
MRD-112, 134
mouse LD_{50}, 134
Mucicarmine-positive renal casts, 50
Mucicarmine stain for *C. neoformans*, 116, 117, 160
Mucosal lesions, 29
Mucous membrane as source of entry, 7
infection with *C. neoformans*, 82
Multiple myeloma, treatment
with propamidine, 130
with 2-hydroxystilbamidine, 131
Musarin, 138
Mycological agar, 101, 152
Mycophil agar, 101, 152
for chromogenesis in fungi, 152
Mycostatin (*see* Nystatin)
Mycosubtilin, 136, 137
Mycoticin, 138, 148
chemical characteristics, 148
effect on *Blastomyces dermatitidis*, 148
effect on *C. neoformans*, 148
effect on *Coccidioides immitis*, 148
effect on *Sporotrichum schenckii*, 148
from *Streptomyces ruber*, 148
toxicity to mice, 148
Myelin globules in sputum, 97
Myocarditis due to *C. neoformans*, 68
Myvisone, effect on *C. neoformans*, 134
Myxomatous-like cryptococcal masses, 25, 54, 58, 65, 66, 67, 82

N

Naphthoquinones
failure to protect mice, 135
for treatment of superficial mycoses, 134
fungistatic properties, 134
Naturally occurring infections, 7
Necrosis in pulmonary tissue, 18
Needle biopsy for diagnosis, 92
N-butylmaleimide, 135
Neoarsphenamine, 135
Neoformans, species term, 1
Neonatal cryptococcosis, 8
pathology, 80
Neopyrithiamine, 149
Nicotinamide, 135
Nitrofurazone (Furacin®), 135
Nitro furfuryl methyl ether, effect on *C. neoformans*, 134
Nitrogen assimilation
agar, 156
broth technic, 107, 156
by *C. neoformans*, 106
by yeasts, 106
Nitrogen auxanographic agar method, 106
North American blastomycosis, treatment
with pentamidine, 132
with phenamidine, 132
with propamidine, 130, 132
with stilbamidine, 130, 132
with 2 hydroxystilbamidine, 131, 132
N^1-p-nitrobenzoyl sulfanilamide, 129
Nystatin, 126, 138, 139, 141, 148
beneficial effect, 149
blood levels, 149
chemical characteristics, 148
effect of blood on activity, 148
effect on *C. neoformans*, 138, 139
effect of cysteine, 139
effect of fatty acids, 139
for coccidioidomycosis, 149
for cryptococcal meningitis, 148
for human cryptococcosis, 37, 126, 139, 148
for mouse cryptococcosis, 139
fungicidal action, 139
intravenous administration, 139, 149
in vitro assay, 148
LD_{50} in mouse, 138
oral use in humans, 139, 149
parenteral toxicity, 148
reversal of activity, 139
sclerosing effect on veins, 149
serum level in guinea pig, 141
spinal fluid levels, 148
stability, 148
tetraene, 147
toxicity for animals, 138, 141
toxicity for humans, 139, 148, 149

O

Ocular cryptococcosis
in cerebral cryptococcosis, 18, 19
in human, 19, 35, 64, 85
in rabbit, 115
Optic nerve, invasion by *C. neoformans*, 20, 34
Osseous cryptococcosis (see Cryptococcosis of bone)
Osteomyelitis, cryptococcal, 28, 31, 32, 33
of humerus, 31, 32

Overdiagnosis, ***115***, 117, 118, ***118***, ***119***
  (see also Russell bodies, Mineral concretions)
Oxophenarsine hydrochloride, 126
Oxygen requirement of *C. neoformans*, 108

P

Papilledema
  in CNS cryptococcosis, 18
  in meningoencephalitis, ***19***
Para-aminobenzoic acid, 135
Para-aminohippuric acid, 135
Para-aminosalicylic acid, 126
Para-hydroxybenzoic acid esters, 133
  effect on *C. neoformans*, 133
  inhibitory to *Candida*, 133
  toxicity, 133
Para-hydroxymethyl benzoate, 133
Para-nitrobenzoic acid, 135
Pasteurization, effect on *C. neoformans*, 7, 45
Pathogenesis of cryptococcosis, 38, 39
Pathogenicity of *C. neoformans*, 111
Pathology of cryptococcosis, 53–90
  abscess formation, 54
  absence of caseation necrosis, 54, 70
  absence of humoral response, 53
  absence of pseudoepitheliomatous hyperplasia, 60
  calcification, 61
  cavitation, 54
  concretions in calcified lesions, 61
  fibrovascular proliferation, 58
  [illegible]
  inflammatory response, 53
  influence of age, race and sex, 61
  influence of lymphoma, 61
  myxomata like lesions, ***25***, 54, ***58***
  of adrenals, ***86***, 87, 89
  of bone, 87, 88
  of bone marrow, 89
  of brain, in encephalitis, 74
  of brain, in meningitis, 71
  of brain, in space occupying granuloma, 82
  of eye, 87
  of kidney, ***86***, 88
  of liver, ***86***, 89
  of lungs, 64
  of skin and mucous membrane, 82
  of soft tissues, 82
  of spleen, ***87***, 89
  polymorphonuclear reaction, 54
  polyp-like lesions, 54
  rarity of fibrous encapsulation, 60
  rarity of necrosis, 54
  sarcoid pattern, 56, ***57***, ***60***
  tissue caseation, 56
  types of lesions, 61
  variation in cellular response, 53
  variation in tissue reaction, 56
Peaches, isolation of *C. neoformans*, 6
  source of *Saccharomyces neoformans*, 1, 2
Pelargonic acid, 134
Pelvis involvement, 31
Penicillic acid, effect on *C. neoformans*, 136
Penicillin in cryptococcosis, 126, 127, 136
  effect on *C. neoformans*, 136
Penicillin G, effect on *C. neoformans*, 136
Penicillin K, effect on *C. neoformans*, 136
Penicillium citrinum, 137
Pentaenes, 147
Pentamidine, 132
Peribronchial infiltration, 14, ***15***
Periodic acid-Schiff stain, ***116***, 117, 158
Perivascular extension in brain, ***55***
pH, optimal for fungi, 127
Phalanx involvement with *C. neoformans*, ***28***
Pharyngeal structures as source of entry, 7
Phenamidine, 132
Phenazine di-N-oxide, 135
Phenothiazine, 135
Physical agents in cryptococcosis, 125, 126
Physiological properties of *C. neoformans*, 105
Pigeons
  excreta, as source of *C. neoformans*, 7
  nests, 7
  relation to C. neoformans, 7
Pitfalls in spinal fluid examination, 99
Pityrosporum genus, 108, 109
Plant extracts effective against *C. neoformans*
  buttercup extract, 143
  Coptis chinensis extract, 144
  garlic clove extract, 144
  Plumbago europa extract, 144
  Spanish moss extract, 144
  tomato extract, 144
  Western red cedar extract, 144
  wheat flour extract, 144
Pleural effusion, ***11***
Pleural granuloma, 11
Pleural reaction, ***10***
Pleural thickening, ***14***
Pleuritis in cryptococcosis, 69
Pleurotin, effect on *C. neoformans*, 136, 137
  mouse toxicity, 137
Pleurotus griseus, 137
Plumbago europa, 144
Pneumonectomy for cryptococcosis, 122, 123
Pneumonitis, ***57***
  relation to pigeon manure, 7

Polarization of light, effect on *C. neoformans*, *116*
Polyene reaction, 147
Polyenes, 147
Polymyxin
  in cryptococcal meningitis, 137, 142, 146
  in cryptococcosis, 126, 127, 136, 142, 145, 146
  mouse LD_{50}, 136
  synergistic action with Actidione, 142
Porcine cryptococcosis, 38
Portal of entry of *C. neoformans*
  gastro-intestinal tract, 6, 34, 39, *39*, 151
  mucous membrane, 7
  pharyngeal structures, 7
  respiratory tract, 7, 8
  skin, 7
Potassium nitrate assimilation
  of *C. neoformans*, 106
  of *C. neoformans* var. *innocuous*, 106
  medium, 156
Precipitin reaction in cryptococcosis, 48
Pregl's iodine solution, 126
Primary inoculation cryptococcosis, 7
Prodigiosin in cryptococcosis, 126, 136, 137, 141
  in cryptococcal meningitis, 137
  mouse toxicity, 136
Promin in cryptococcosis, 126
Protoanemonin
  chemical nature, 143
  effect on *C. neoformans*, 143
  for cryptococcal meningitis, 143
  for osseous cryptococcosis, 143
  mouse toxicity, 143
  source, 143
  toxicity in humans, 143
Propamidine, 130, 132, 135
  effect on *C. neoformans*, 132
  failure to protect mice, 132
  for treatment of kala-azar, 130
  for treatment of multiple myeloma, 130
Protoanemonin in cryptococcosis, 126, 136, 143
  effect on *C. neoformans*, 136
  mouse toxicity, 143
Pseudomethyl acetylacrylate, effect on *C. neoformans*, 159
Pseudomonas aeruginosa, 136
Pseudomycelium in *C. neoformans*
  in culture, 95, *95*, *98*
  in tissue, 87
Pulmonary calcification, 63
Pulmonary consolidation, *14*, *18*, *66*
Pulmonary cryptococcosis (see Cryptococcosis of lung)
Pulmonary resection for cryptococcosis, 122
Purified agar, 107
Purothionin, 144
  mouse toxicity, 144
Pyocyanine, 136, 137
Pyribenzamine, 126, 135
  effect on *C. neoformans*, 135
Pyridine for complement-fixing antigen, 151
Pyrimidine, 135
Pyrithiamine, 149

Q

Quellung reaction with *C. neoformans*, *49*, 50, 99
Quinacrine
  effect on *C. neoformans*, 135
  serum concentration, 135
Quinine, 135

R

Rabbit cryptococcosis, *115*
Rabbit, susceptibility to *C. neoformans*, 114
  susceptibility, anterior chamber, 114, *115*
Racial differences in cryptococcosis, 7
Radiologic characteristics of pulmonary form (see Cryptococcosis of lungs)
Radius involvement with *C. neoformans*, 32
Ranunculaceae plant extracts, 143
Rat, susceptibility to *C. neoformans*, 113
Relation of pneumonitis to pigeon manure, 7
Remission (see Spontaneous remission in cryptococcosis)
Renal cryptococcosis, 17, 34, *86*, *90*, *93*
  excretion of cryptococcal polysaccharide, *90*
Reproduction in yeasts, 103
Resorcinol, 135
Respiratory tract as portal of entry of *C. neoformans*, 7
Reticulum structure, effect of cryptococcosis, *70*
Retinal invasion, *64*, *85*
Rheumatic heart disease, due to *C. neoformans*, 151
Rhinehart-Abul-Haj stain, 117
  (see also Acid mucopolysaccharide stain)
Rhinosporidium seeberi, mucicarmine stain, 117
Rhizoctonia factor, 137
Rhodotorula genus, 108, 109
Rhodotorula glutinis, 108
Rhodotoruloideae subfamily, 108, 109
Rimocidin, 138, 141, 147
  mouse LD_{50}, 138
  toxicity, 138, 141
Rodent cryptococcosis, 112, 113, *113*

Rodent ulcer-like granuloma, due to *C neoformans*, 27
Roentgen diagnosis in pulmonary cryptococcosis (see Cryptococcosis of lungs)
Roentgen therapy in cryptococcosis, 125, 126, 127, 128
effect on CNS lesions, 128
effect on *C neoformans*, 128
effect on cutaneous lesions, 128
effect on nasopharyngeal lesions, 128
effect on pulmonary lesions, 128
favorable results, 128
Roentgenograms
calcification, 16
caseation necrosis, 14
cavitation, 14, 15, 16
differentiation from other pulmonary diseases, 15
diffuse infiltration, 14
fibrosis, 14, 16
important radiologic characteristics, 16
miliary lesions, 14
of bones and joints, *28, 31, 32, 33, 35*
differentiation from other mycoses, 34
radiologic characteristics, 34
of humerus, *31, 32*
of ischium, *33*
of lung in cryptococcosis, *10, 11, 12, 13, 14, 16, 17, 18*
of phalanx, *28*
of radius, *32*
of skull, 21
of tibia, *33*
of tibial tubercle, 33
peribronchial infiltration, 14
rarity of coin lesions, 15
solitary infiltration, 13
RO-2-3094, 129
mouse LD_{50}, 129
Russell bodies, *115*, 118, *119*
Rutin, reversing effect on tomatin, 144

S

Sabouraud dextrose agar, 101, 152
appearance of *C neoformans*, *100, 104*
appearance of *C neoformans* var *innocuous*, *104*
effect of thiamine, 152
formula, 152
for stock cultures, 152
incorporation of antibiotics, 152
Saccharomyces, 1
Saccharomyces blanchardi, 111
Saccharomyces breweri, 111
Saccharomyces cereviseae, effect of compound P-594, 134
Saccharomyces hominis, 111
Saccharomyces lithogenes, 110
Saccharomyces neoformans, 1, 110
Saccharomyces pastorianus, in Actidione assay, 140
Saccharomyces plimmeri, 111
Saccharomyces subcutaneus tumefaciens, 1
Saccharomycosis hominis, 1
Sarcoid-like pattern in cryptococcosis, 56, 57, *69*
Sarcoidosis, relation to cryptococcosis, 36
similarity to cryptococcosis, *17, 60*
Schiff's reagent, 158
Sectoring of *C. neoformans* colony, *104*
Serologic grouping of *C. neoformans*, 47, 49
Serratia marcescens, 141
Silver mercaptoacetyl sulfathiazole, 129
Silver nitrate stain, 161
Simian cryptococcosis, 38, 39
Siosteran, 150
6-cyano-2-methoxy-9-isopropylaminopropylaminoacridine, 135
6-methoxy-8-isopropyl-amino-pentylaminoquinoline, 135
6-methoxy-8-morpholino-propylamino quinoline, 135
6-phenyl-5,6-dihydrouracil, 135
Skin, habitat for cryptococci, 6
as portal of entry, 7
Skin hypersensitivity in mycotic disease, 47, 51
in cryptococcosis, 51
Skin test in cryptococcosis, 51, 52
with boiled extract, 51
with broth filtrate, 51
with HCL treated suspensions, 52
with saline suspensions, 51
with torulin, 52
Smallpox-like cutaneous cryptococcal lesions, *27*
Sodium bicarbonate, 126, 127
Sodium bismuth mercaptoacetyl sulfathiazole, 129
Sodium-5-sulfanilamido-naphthalene-1-sulfonate, 129
sodium p,p'diaminodiphlenylsulfone-N,N'-didextrose sulfonate (Promin®), 135
Sodium sulfanilyl-sulfanilate, 129
Sodium thiosulfate in cryptococcosis, 126
Sodium-2-sulfanilamido benzoate, 129
Soil, source of *C neoformans*, 7
Soluble cryptococcal antigen, 48, 50
in blood, 48
in humans, 48
in mice, 50
in spinal fluid, 48
in urine, 50

Source of infection of *C neoformans*, 6
Space-occupying granuloma, in brain, 82
Spanish moss, 144
(see also *Tillandsia usneoides*)
Spinal fluid, in CNS cryptococcosis, 91, 92, 96
chemical changes, 92
dynamics, 20
method of examination, 96
physical changes, 91
Splenic cryptococcosis, *87*, 89
Spontaneous infection in animals, 38, 39, 40
air-borne infection, 39
alimentary tract, 39, *39*, *40*
facial lesions, *38*, 39
nervous system involvement, *40*, 40
pulmonary involvement, 39
Spontaneous remission in cryptococcosis, 19, 121, 123, 125
Sporotrichum schenckii
effect of mycoticin, 148
effect of propamidine, 130
mucicarmine stain, 117
Sporulation agar for yeasts, 157
Sputum examination, in cryptococcosis, 16, 91, 96, *97*
artefacts, *97*
diagnosis, 16, 91, 96
Starch production by *C neoformans*, 107
culture medium, 157
Stibophen (Fuadin®), 135
Stilbamidine, 126, 128, 130, 131, 132, 140, *141*
failure with cryptococcal-infected mice, 132
for pulmonary blastomycosis in mice, 130
for treatment of kala-azar, 130
for treatment of multiple myeloma, 130
mode of administration, 131
mouse LD_{50}, 141
pharmacology, 131
serum level in guinea pig, 140, 141
structural formula, 131
toxicity, 130, 141
Strawberry-like cryptococcal lesions of skin, *24*
Streak plate method for assimilation tests, 107
Streptodornase in cryptococcosis, 126, 127, 142, 145, 146
in cryptococcal meningitis, 142, 146
Streptokinase, 145
Streptomyces (WC 3569), 138
Streptomyces albus, 138
Streptomyces antibioticus, 138
Streptomyces canescus, 138, 139, 141
Streptomyces cellulosae, 138, 147
source of fungichromin, 147
Streptomyces fradiae, 138
Streptomyces griseus, 138, 139, 141
Streptomyces lavendulae, 138
Streptomyces noursei, 138, 139, 141
Streptomyces rimosus, 138, 141
Streptomyces ruber, 138, 148
source of mycoticin, 148
Streptomyces sp, 138
Streptomyces viridoflavus, 138
Streptomycin in cryptococcosis, 126, 127, 136, 138
effect on *C neoformans*, 136, 138
mouse LD_{50}, 138
toxicity, 138
Streptothricin, 138
mouse LD_{50}, 138
toxicity, 138
Subcutaneous lesions, 25
Subpleural granuloma, 8, *9*, 11, *53*, *60*, *61*, *64*, 68
pathology, 68
Subtilin, effect on C neoformans, 136
Sulfadiazine in cryptococcosis, 126, 127, 129, 130, 145
blood levels, 130
curative effect, 130
spinal fluid level, 130
Sulfamerazine in cryptococcosis, 126, 129
effect of blood on activity, 129
Sulfanilamide in cryptococcosis, 126, 129, 130
blood levels, 130
Sulfanilamido-acetic acid, 129
Sulfapyridine in cryptococcosis, 126, 130
blood levels, 130
spinal fluid level, 130
Sulfathiazole in cryptococcosis, 126, 129
Sulfonamides in cryptococcosis, 126, 129, 130, 146
in-vitro resistance of *C neoformans*, 129
leukopenic effect, 130
Summary of treatment, 146
Surgical treatment of cryptococcosis, 20, 21, 121
"cure," 21
of cerebral form, 20
of osseus cryptococcosis, 31
Susceptibility to cryptococcosis, 7
race differences, 7
Symptomatic treatment, 146
lumbar puncture, 146
of ambulatory patient, 146
of hospitalized patient, 146
opiates, 146
Symptomatology, mastitis of cattle, 41, 42, *42*
Synkamin, effect on *C neoformans*, 150

Synonymy of *C. neoformans*, 3, 110
Synthetic agar for extracellular starch, 157

T

Temperature of experimental animals, 125, 127
Temperature tolerance of *C. neoformans* var. *innocuous*, 104
Terramycin in cryptococcosis, 126, 136
Testicular cryptococcosis, *89*
Thiamin in cryptococcosis, 126
  effect on *C. neoformans*, 100, 149
Thiocarbamate derivatives, 134
  effect on *C. neoformans*, 134
  failure to protect mice, 134
Thiocarbamyl hydrazine, 134
  effect on *C. neoformans*, 134
Thiolutin, 138
  mouse LD_{50}, 138
  toxicity, 138
Thiosemicarbazones, effect on *C. neoformans*, 133
Thiourea derivatives, 134
  effect on *C. neoformans*, 134
Thoracentesis in cryptococcosis, 12
  for diagnosis, 12
3,5'-dibromosulfanilanilide, 129
3-phenyl hydantoin, 135
Thuja plicata, 144
Thymol in cryptococcosis, 126
Tibial involvement due to *C. neoformans*, *33*
Tibione, effect on *C. neoformans*, 134
Tillandsia usneoides, 144
Tissue artefacts, 117
Tissue response in animals, 40, ***40***, ***41***
Toluidine blue stain for *C. neoformans*, 99, ***116***
Tomatin, 114
  reversing effect of rutin, 144
Tomatine, 144
Tomato extracts, 144
  effect on *C. albicans*, 144
Torula histolytica, 1, 2, 111
Torula kleini, 111
Torula nasalis, 111
Torula neoformans, 110
Torula plimmeri, 111
Torulin for skin test, 52
Torulopsis breweri, 111
Torulopsis costantini, 111
Torulopsis genus, 108, 109
Torulopsis glabrata, 111
  from human sources, 111
  simulating histoplasmosis, 111
Torulopsis histolytica, 111
Torulopsis hominis, 111
Torulopsis hominis var. *honduriana*, 111
Torulopsis lithogenes, 111
Torulopsis neoformans, 110
Torulopsis neoformans, var. *sheppei*, 111
Torulopsis plimmeri, 111
Torulosis, 1
Toxoplasma gondii infection, misclassified, 80
Transmission of cryptococcosis, 7
Treatment of cryptococcosis (see Cryptococcosis, treatment)
Trichoderma, 137
Trichoderma viride, 137
Trichosporoideae subfamily, 108, 109
Trichosporon cutaneum, 108
Trichosporon genus, 108, 109
Trichothecin, 137
Trichothecium roseum, 137
[illegible]
2-bromo-1-methoxy-2-nitro-1-phenylpropane, 134
2-(dimethylaminomethyl)-1-naphthol hydrochloride, 135
2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine, (see Daraprim)
2 hydroxystilbamidine, 126, 128, 130, 131, 132, 146
  effect on *B. dermatitidis*, 131, 132
  effect on *H. capsulatum*, 131
  for cryptococcosis, 126, 128, 130, 132, 146
  for multiple myeloma, 130
  for North American blastomycosis, 131
  pharmacology, 131
  toxicity, 131
2-metanilamido-5-chloropyrimidine, 135
2-methyl-5-hydroxy-1,4-naphthoquinone, 144
2,3-dichloro-1,4-naphthoquinone, 134
Typhoid-paratyphoid vaccine, 126, 127, 145
Tyrocidin, 126, 136
  effect on *C. neoformans*, 136
  in cryptococcosis, 126, 136

U

Ultra violet radiation in cryptococcosis, 126
Undecylenic acid, 109
Urease formation by *C. neoformans*, 108
Urine culture, in cryptococcosis, 92, 93, 151
  diagnostic potentialities, 34
Uronic acid, in cryptococcal polysaccharide, *48*
Ustilagic acid, 137
  mouse toxicity, 137
Ustilago zeae, 137
Uveal tract of eye, cryptococcal lesion, *35*

V

Vaccine therapy in cryptococcosis, 126, 145, 146
desensitization procedure, 145
in cryptococcal meningitis, 145
in osseous cryptococcosis, 145
routes of administration, 145
skin reactions in cryptococcal meningitis, 145
Vacuolization in tissues, *57*
Vanithiazone, effect on C. *neoformans*, 104
Ventricular dilation in cryptococcosis, *21*, *79*, 80, *80*
Vertebral involvement in cryptococcosis, 31
Violacein, 136, 137
Viridin, 137
Virulence of C. *neoformans*, 111
enhancement by serial passage, 111

W

Washed agar, 107, 155
Western red cedar extract, 144
mouse toxicity, 144
Wheat flour extract, 144
mouse toxicity, 144
Wisconsin enzootic in cattle, 42

X

X-ray therapy (see Roentgen therapy)
Xylose, in cryptococcal polysaccharide, 48

Y

Yeast carbon base, 156
Yeast morphology agar, 101, 154
Yeast nitrogen base, 155